NON-RECIPROCAL CROSS-STERILITY IN MAIZE[1]

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THE first record of the inability of a variety of maize to set seed when pollinated by pollen of another variety was made by DEMEREC (1929). In the course of genetic investigations, he found that plants of a family of White Rice popcorn were not producing seed when pollinated by foreign pollen. However, the reciprocal crosses (in which the White Rice plants were used as male parents) always gave a full set of seed. It should be noted that although DEMEREC refers to the variety with which he worked as White Rice, his photographs show it to be a White Pearl type, which is quite distinct from White Rice. It will be referred to hereafter as Rice (Pearl). BRUNSON (1937) found an analogous situation with regard to the popcorn varieties, Supergold and South American. Attempts to cross the two varieties were unsuccessful when Supergold was used as the pollen parent, but the cross could be made without difficulty when South American was used as the male parent.

In his analysis, DEMEREC showed that the F_2 generations of crosses of various sweet corns with Rice (Pearl) had marked deficiencies of sugary kernels—12.4 percent instead of the expected 25 percent. Therefore, he assumed a relationship between the cross-sterility of Rice (Pearl) popcorn and the dominant gametophyte factor *Ga* which had been reported by EMERSON (1925) and MANGELSDORF and JONES (1926). *Ga* is on chromosome 4 and linked to the *Su* locus. Here, too, there is a deficiency of sugary kernels in the F_2 of the cross *ga su/ga su* × *Ga Su/Ga Su*—EMERSON reporting 15.4 percent sugary kernels, and MANGELSDORF and JONES 16.2 percent sugary kernels. The latter situation is somewhat different since plants which are *Ga/Ga* will set seed readily when pollinated by *ga* pollen. The effect of the gametophyte factor is operative only when *ga* and *Ga* pollen are competing on silks which are *Ga/Ga* or *Ga/ga*. In this case, EMERSON (1934) has estimated that on the average only 4 percent *ga* gametes effect fertilization. It is obvious that a gametophyte factor of this type is detectable only when a linked mutant gene is segregating and then by the excess or deficiency of the expected mutant types depending on whether it is linked to *Ga* or *ga*. When the mutant gene is linked to *ga* as *su* is in the case above, the great majority of the gametes carrying *su* which effect fertilization are those in which a crossover has occurred between *su* and *ga* so that *su* is carried in a *Ga* gamete. Using 4 percent as the amount of *ga* pollen effecting fertilization on *Ga/ga* styles when in competition with *Ga* pollen EMERSON estimated 28 percent recom-

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bination between *su* and *ga*. The deficiency of sugary kernels in the F_2 generation of a cross of a sweet corn by a line carrying the gametophyte factor is thus due to *su* entering the cross linked to *ga*. In interpreting their results, MANGELSDORF and JONES assumed the mechanism to be differential pollen-tube growth such that *ga* gametes rarely achieved fertilization when competing with *Ga* gametes on *Ga/ga* or *Ga/Ga* silks. DEMEREC felt that his results ruled out this explanation since in his stock of Rice (Pearl) popcorn, foreign pollen only rarely induced any seed set. Here competition between gametes by virtue of different growth rates could not be the cause.

SCHWARTZ (1950) recently has supplied the means of reconciling the observations of DEMEREC with those of MANGELSDORF and JONES, and EMERSON. He has analyzed a case of cross-sterility in maize which acts in the same fashion as the cross-sterility reported by DEMEREC, *i.e.*, a line which is sterile to foreign pollen but which itself can fertilize any other line and in which the F_2 generation of a cross on sugary has a marked deficiency of sugary kernels (12.9 percent). SCHWARTZ showed that this was due to the existence at the *ga* locus of a third allele which he has designated Ga^s because its action is stronger than *Ga*. SCHWARTZ has estimated 25 percent recombination between *Su* and Ga^s.

At the locus in question there are then three alleles, *ga*, *Ga*, and Ga^s. The *ga* allele is carried by most varieties of corn. *Ga/Ga* plants can be fertilized by *ga* pollen when this pollen is not competing with *Ga* pollen. Ga^s/Ga^s plants do not set seed when pollinated by *ga* pollen even in the absence of competing gametes but do set readily when pollinated by *Ga* pollen. On Ga^s/Ga silks Ga^s pollen has a slight competitive advantage over *Ga* pollen. On Ga^s/ga silks Ga^s pollen has a competitive advantage of almost 1 over *ga* pollen. Plants which are *ga/ga* show perfect seed sets when pollinated by either *Ga* or Ga^s pollen. Furthermore, on styles of this genotype (*ga/ga*) neither Ga^s nor *Ga* pollen has a competitive advantage over *ga* pollen.

DEMEREC pointed out the connection between cross-sterility in Rice (Pearl) popcorn and the *Ga* locus. SCHWARTZ has shown that a factor for cross-sterility is an allele at that locus and has suggested that other cases of cross-sterility might have a similar genetic basis.

CROSS-STERILITY IN POPCORN

For the past four years we have been investigating the extent of cross-sterility among popcorn varieties as well as following the inheritance of cross-incompatibility directly rather than by the disturbance of the expected Mendelian ratios of genes linked with the locus responsible. In the initial stage of the investigation, a crossing block was set up with ten inbred lines, and all possible crosses were made reciprocally between the ten inbreds. The ten inbreds were SA24 from South American popcorn, 1001–52 from Hulless popcorn, 4541–U from Black Beauty popcorn, 845–1B from Ohio Yellow popcorn, 4524–4 from Red popcorn, A3–1 from Amber Pearl popcorn, 1708–4 from Baby Golden–1 popcorn, Sg18 from Supergold popcorn, and Hy from Illinois High Yield dent corn.

All pollinations were made early in the morning on silks which had been cut back to the tip of the cob the previous day. At harvest time, the crossed ears were scored for extent of seed set. Those crosses in which seed set was less than 10 percent normal were regarded as incompatible. Those in which the seed set was greater than 90 percent of normal were regarded as completely compatible while intermediates were scored according to the percentage of seed set. When seed set is intermediate, it is common to find that almost all the kernels are concentrated in the upper third to half of the ear. In sterile crosses where one to a few kernels are found, they are usually on the tip of the ear.

These results which are summarized in table 1 are the average of four or five individual pollinations. Here it is obvious that the ten inbreds fall into three groups. The first (comprising SA24, 1001–52, 4541–U, 845–1B, 4524–4 and A3–1) is sterile or partly sterile with pollen from Hy, Sg18 and 1708–4. The inbreds in this group pollinate any other inbred. The second class (with 4519–41 only) sets seed with any other inbred and fertilizes any other inbred. The third group (1708–4, Sg18, and Hy) sets seed with pollen from any other inbred but does not induce seed set in inbreds of the first group. It should also be noted the incompatibility is not necessarily of an all-or-none type. In some cases such as 4541–U × 1708–4 or 4524–4 × Sg18 the seed set is clearly less than normal but more than that found in some crosses where none to a very few kernels are found per ear.

For ease in future discussion, inbreds of the first group (SA24, etc.) will hereafter be referred to as cross-sterile, inbreds of the second group (4519) as cross-neutral, and those of the third group (Hy, etc.) as normal since their reaction is that typical of most varieties of corn. Also plants that set seed with dent corn pollen will be designated as receptive.

In 1949 a similar all-combination crossing block was set up with some inbreds being dropped and still others being added. At the suggestion of DR. M. M. RHOADES, Ga^s/Ga^s and *Ga su/Ga su* were added to the test. These stocks were obtained from DR. DREW SCHWARTZ. An inbred from the popcorn variety, Yarling Yellow, 1491–KA, was also added. The same procedure was followed as in the previous year, but pollinations were made at noon or late rather than early in the morning. The results again are summarized in table 1. Many crosses which gave unambiguous reactions in 1948 were not repeated in 1949. It is interesting to note that all the deviations of 1949 results from 1948 results are in the same direction—towards less seed set. Some crosses which had given a normal seed set in 1948 showed only partial seed sets in 1949 while others which had partial seed sets in 1948 were completely sterile in 1949. This is a clear demonstration of the fact that environmental conditions can play a decisive role in their influence on incompatibility reactions and a corollary of the previously noted fact that cross-sterility in many cases may be intermediate instead of absolute.

Further, it is apparent that Ga^s/Ga^s, which is SCHWARTZ's super-gametophyte factor discussed previously, acts in a test of this sort exactly as do the cross-sterile popcorns. It will induce setting of seed in any other inbred but

TABLE 1

Degree of seed set in reciprocal pollination tests of thirteen inbreds, 1948 and 1949*

Female	Male												
	1948		1948 and 1949								1949		
	White Rice 4519-41	Ohio Yellow 845-1B	Amber Pearl A3-1	South American SA24	Hulless 1001-52	Black Beauty 4541-U	Red 4524-4	Baby Golden-1 1708-4	Supergold Sg18	Dent Hy	Yarling Yellow 1491-K	*Ga/Ga*	*Ga^s/Ga^s*
1948													
4519-41	+	+	+	+	+	+	+	+	+	+			
845-1B	+	+	+	+	+	+	+	−	−	−			
1948 and 1949													
A3-1	+	+	+	+	+	+	+	+	.4(−)	.4(−)	+		
SA24	+	+	+	+	+	+	+	+(.25)	.5(−)	−−	+	+	+
1001-52	+	+	+	+	+	+	+	−	−−	−−		+	+
4541-U	+	+	+	+	+	+	+	.5	−	−	+		
4524-4	+	+	+	+	+	+	+	.6(−)	.2(−)	.1(−)	+	+	+
1708-4	+	+	+	+	+	+	+	+	+(.4)	++	+		
Sg18	+	+	+	+	+	+	+	+	+	+			+
Hy	+	+	+	+	+	+	+	+	+	+	+	+	+
1949													
1491					+	+	+	.3	.2	.2	+	+	+
Ga/Ga												+	
Ga^s/Ga^s				+	+	+	+		−	−		+	+

*A "+" indicates full seed set; a "−" indicates less than 10% seed set; a decimal indicates proportionate seed set. Two values for any cross means that the cross was made in both 1948 and 1949. Where the 1949 result differs from the 1948 cross, it is enclosed in parenthesis.

fails to set seed with pollen from Sg18 and Hy. It sets seed fully with all the cross-sterile popcorns which were used as males, and the cross-sterile popcorns set seed with its pollen. This indicates that the factor for cross-sterility carried by SA24, 1001–52, 4541–U and 4524–4 may be identical with *Ga*^{s}. A third, reciprocal, all-combination, crossing block in 1950 has given essentially the same results and reinforced our conclusions that *Ga*^{s}/*Ga*^{s} and the cross-sterile popcorn inbreds are alike in test crosses and that in cases of partial cross-sterility the degree of seed set can vary rather widely from year to year.

DIRECT TESTS OF THE INHERITANCE OF CROSS-STERILITY

Indications that the cross-sterile popcorns are indistinguishable from *Ga*^{s}/*Ga*^{s} in test crosses make it highly probable that their inability to set seed with dent corn pollen is conditioned by an allele at the 4th chromosome locus. Further evidence is the fact that the F_1 cross of the cross-sterile popcorn SA24 times *Ga*^{s}/*Ga*^{s} does not set seed with dent corn pollen. And the percentages of sugary kernels in the F_2 progeny of crosses of *ga su*/*ga su* × the cross-sterile popcorns vary from 13.9 to 15.5 which values are between that found by SCHWARTZ (1950) for the F_2 of a cross of *ga su*/*ga su* × *Ga*^{s} *Su*/*Ga*^{s} *Su* (12.9) and that found by EMERSON (1934) in the cross of *ga su*/*ga su* × *Ga Su*/*Ga Su* (15.4). In the F_2 of the cross of SA24 times *Ga*^{s}/*Ga*^{s} there were, however, 6 partially receptive and 74 non-receptive plants. The partially receptive plants have been interpreted as being due to the segregation of modifying genes which will later be shown to be crucial in cross-sterility reactions. A possible alternative interpretation is that popcorn cross-sterility is due to a second gametophyte factor, *Ga*$_x$, lying equidistant from *su* and the other side of *su* from *Ga*^{s}. Then the appearance of partially receptive plants in the F_2 of the above cross is due to crossing-over which puts *ga* and *ga*$_x$ in the same gamete. Such recombination would be nearly at random. Male gametes that were *ga ga*$_x$ would not be effective competitively and can be disregarded. A quarter of the female gametes would be *ga ga*$_x$ and give rise to partially receptive plants regardless of the constitution of the male gamete. This is significantly more than are found. Further, for popcorn cross-sterility to be due to this hypothetical second locus, *Ga*$_x$, would entail certain assumptions. These assumptions are (1) that *Ga*^{s} *ga*$_x$/*Ga*^{s} *ga*$_x$ plants and *ga Ga*$_x$/*ga Ga*$_x$ plants would have the same phenotype (no seed set with dent corn pollen), (2) that both *Ga*^{s} *ga*$_x$ and *ga Ga*$_x$ gametes would effect fertilization when growing in either *Ga*^{s} *ga*$_x$/*Ga*^{s} *ga*$_x$ or *ga Ga*$_x$/*ga Ga*$_x$ silks, and (3) that while *ga ga*$_x$/*ga Ga*$_x$ plants would be cross-fertile, *Ga*^{s} *ga*$_x$/*ga Ga*$_x$ plants would be cross-sterile. In particular the second seems to be unlikely, and all three taken together generate a high order of improbability. On the basis of available evidence it seems very likely that the genetic basis of cross-sterility in the popcorns is *Ga*^{s} or an allele much like it, and further discussion will be conducted on this premise. In this case, the cross-sterile popcorns would themselves be *Ga*^{s}/*Ga*^{s} while the cross-fertile popcorns and the dent inbred Hy

would be *ga/ga*. The White Rice inbred, 4519–41, which does not participate in any sterility reaction on either the male or female side, would be *Ga/Ga*. If so, there are grounds for predicting the phenotypes in backcrosses of (normal × cross-sterile) × cross-sterile and in the F_2 progenies of (normal × cross-sterile). In most instances, the F_1 progeny of a cross of normal × cross-sterile will set seed readily with dent corn (*ga*) pollen so plants heterozygous for the gene conditioning cross-sterility show a normal phenotype. It should be noted here that the gene symbolism employed is confusing. Originally, the allelic pair *Ga* and *ga* were so designated because when competing on *Ga/ga* silks *Ga* gametes effect fertilization almost to the exclusion of *ga* gametes. Hence the symbols indicate not the dominance relationship of the alleles but their competitive effectiveness in the gametophyte generation. However, the F_1, *ga/Ga*^{s}, as noted, is usually completely fertile with *ga* pollen. Thus *ga* behaves as a dominant so that *ga/ga* plants and *Ga*^{s}*/ga* plants are both receptive to *ga* pollen. Bearing in mind the known competitive aspects of fertilization when *Ga*^{s} and *ga* gametes are used to fertilize *Ga*^{s}*/Ga*^{s} or *Ga*^{s}*/ga* pistils, in the backcrosses we should find two possible types of progenies depending on which way the backcross was made. If it is made *ga/Ga*^{s} × *Ga*^{s}*/Ga*^{s}, then there should be two classes equal in number—those (*Ga*^{s}*/ga*) that will set seed with *ga* pollen and those (*Ga*^{s}*/Ga*^{s}) that will be non-receptive to dent corn pollen. If the backcross is made in a reciprocal fashion, *Ga*^{s}*/Ga*^{s} × *Ga*^{s}*/ga*, there should be only one class in the progeny—plants which are *Ga*^{s}*/Ga*^{s}, and barren when pollinated by *ga* pollen. This is a result of the complete or almost complete elimination of the *ga* gametes when competing with *Ga*^{s} gametes on *Ga*^{s}*/Ga*^{s} pistils.

With regard to the F_2 progenies of these crosses of normal × cross-sterile lines, the expectation also is for a segregation into two equally sized groups—one of which (*Ga*^{s}*/ga*) will accept dent corn pollen and the other of which (*Ga*^{s}*/Ga*^{s}) will not. This again is a consequence of the inability of *ga* to effect fertilization when in competition with *Ga*^{s} gametes on *Ga*^{s}*/ga* silks.

In making large scale tests of the phenotypes of the backcross and F_2 progenies detasseling blocks with dent corn pollinators were set up to eliminate the necessity of hand pollinating large numbers of plants. The detasselling blocks were carefully isolated from any source of popcorn pollen, but no attempt was made to secure isolation from dent corn fields since our tests have shown that no dent corn tested is capable of pollinating any cross-sterile popcorn. The pollinators were the dent corn hybrids U.S. 13 and Ind. 210B planted at several different dates to insure an adequate supply of pollen throughout the silking period of the female rows. The female rows are, of course, detasselled so that the only pollen falling on the silks of the female is from the dent corn pollinators and hence *ga*.

The results of these tests of the backcross progenies of the type *ga/Ga*^{s} × *Ga*^{s}*/Ga*^{s} and *Ga*^{s}*/Ga*^{s} × *ga/Ga*^{s} are given in table 2. In compiling this table from our data, all plants with a seed set of 70 percent or better are counted as receptive; those with a seed set of 50–60 percent are counted as doubtful; and

those with a seed set of 40 percent or less are counted as non-receptive. Comparing item 1 with item 2, and item 3 with item 4 in table 2, it is apparent that whether the single cross or the inbred is used as the female parent in the backcross is critical in determining whether the progeny will contain any individuals capable of setting seed with dent corn pollen. Where the Ga^s/Ga^s inbred is used as the female parent, all the offspring are non-receptive. If the F_1 is used as the female parent, the progeny contains approximately equal classes of acceptors and non-acceptors of dent corn pollen.

TABLE 2

Segregation of backcross progenies into receptive and non-receptive classes on the basis of seed set when pollinated by ga pollen and the percentage of seed set in each parental single cross when pollinated by ga pollen, 1949 and 1950.

Item	Year	Pedigree	F_1 data	BC data			
			Percent seed set in single cross parent	No. plants non-receptive	Doubtful	No. plants receptive	Percent non-receptive*
1	1949	(SA × P20$^+$) × SA	100	228	35	193	54.2
2	1949	SA × (SA × P20$^+$)	100	457	20	4	99.1
3	1950	(16$^+$ × 24) × 24	100	107	3	112	48.9
4	1950	24 × (16$^+$ × 24)	100	183	4	0	100.0
5	1950	(Hy$^+$ × 24) × 24	70	46	1	58	44.2
6	1950	(Hy$^+$ × 1001) × 1001	?	43	7	58	42.6
7	1950	(Hy$^+$ × 4541) × 4541	100	56	8	42	57.1
8	1950	(A3 × Hy$^+$) × A3	?	37	4	41	47.4
9	1950	(Hy$^+$ × 845) × 845	40	86	7	8	89.6
10	1950	(Hy$^+$ × 4524) × 4524	20	77	9	31	71.3
11	1950	(18$^+$ × 845) × 845	100	58	4	43	57.4
12	1950	(845 × 18$^+$) × 845	100	48	1	31	60.8

$^+$*ga/ga* parent. All other parents assumed to be Ga^s/Ga^s.
* Doubtful class omitted in calculating percentages.

If items 1, 3, 5, 6, 7, 8, 11 and 12 are pooled, we find that of a total of 1197 plants, 623 are non-receptive to *ga* pollen while 574 plants are receptive. A chi-square of 2.006 shows this to be in agreement with our expectation of a 1 : 1 segregation.

Items 9 and 10 although the same type of backcross ($ga/Ga^s \times Ga^s/Ga^s$) as items 1, 3, 5–8, 11 and 12 depart widely from expectations with far more non-receptive plants than receptive plants. The explanation for this is probably to be found in the amount of seed set by the F_1 parent (ga/Ga^s) when pollinated by *ga* pollen. It will be noticed that this is only 40 percent for item 9 and 20 percent for item 10. Presumably then in the backcross progeny many heterozygous plants (ga/Ga^s) show little to no seed set and are indistinguishable from Ga^s/Ga^s plants. With the items that show a close approximation to expected ratios the F_1 parents (ga/Ga^s) have a normal seed set when pollinated by *ga* pollen. Then plants of the same genetic constitution in the back-

cross progeny also set seed with *ga* pollen and are distinguishable from their Ga^s/Ga^s sibs.

Table 2 also indicates that there is no cytoplasmic influence involved in this type of cross-sterility. Items 11 and 12 represent the same backcross made on reciprocal single crosses—normal × cross-sterile and cross-sterile × normal —and the segregations in the backcross progenies are almost exactly the same. It is often difficult to obtain much seed of a cross of the type, cross-sterile × normal, but enough can be obtained so that good-sized backcross progenies can be made.

A situation which is much more difficult of analysis is found when the segregation of receptive and non-receptive plants in the F_2's of crosses of normal × cross-sterile (table 3) is considered. Here again the parents pre-

TABLE 3

Segregation of F_2 progenies into receptive and non-receptive classes on the basis of seed set when pollinated by ga pollen and the percentage of seed set in each parental single cross when pollinated by ga pollen, 1949 and 1950.

Item	Year	Pedigree	F_1 data	F_2 data			
			Percent seed set in single cross parent	No. plants non-receptive	Doubtful	No. plants receptive	Percent non-receptive*
1	1949	(P20$^+$ × SA) F_2	100	25	6	168	13.0
2	1949	(SA × P20$^+$) F_2	100	24	15	190	11.2
3	1950	(16$^+$ × 24) F_2	100	145	0	273	34.9
4	1950	(Hy$^+$ × 24) F_2	70	70	3	35	66.7
5	1950	(Hy$^+$ × 1001–52) F_2	?	72	3	44	62.1
6	1950	(Hy$^+$ × 4541) F_2	100	165	9	41	80.1
7	1950	(Hy$^+$ × 845) F_2	40	179	7	38	82.5
8	1950	(Hy$^+$ × 4524) F_2	20	146	7	64	69.5
9	1950	(18$^+$ × 845) F_2	100	56	3	50	52.8
10	1950	(845 × 18$^+$) F_2	100	96	3	95	50.3

$^+$ *ga/ga* parent. All other parents assumed to be Ga^s/Ga^s.
* Doubtful class omitted in calculating percentages.

sumed to be *ga/ga* are P20, Sg16, Hy, and Sg18. The percentage of non-receptive plants varies from 11.2 to 82.5, and in only two instances, items 9 and 10, do the numbers of non-receptive plants approximate those expected on theoretical grounds or one-half. It is remarkable to find that in three crosses—items 4, 5, and 6—the percentage of non-receptive plants in the F_2 is greater than the percentage of such plants in backcross progenies obtained by pollinating the F_1's by the cross-sterile parent (cf. items 5, 6 and 7 in table 2). With two other crosses—items 7 and 8—the percentage of non-receptive plants in the F_2 closely approaches the percentage of non-receptive segregates in the backcross to the cross-sterile parent (cf. items 9 and 10 in table 2). No explanation can be offered for this anomalous behavior at this time.

It is clear from a consideration of the data in table 3 that where the normal parent (*ga/ga*) was an inbred or single cross derived from the popcorn variety, Supergold, such as Sg16, Sg18 or P20 (Sg18 × Sg30A) the percentage of non-receptive plants is equal to or less than the expectation of 50 percent as shown in items 1, 2, 3, 9 and 10. Where the normal parent is the dent corn inbred, Hy, the percentage of non-receptive plants is always greater than 50 percent—items 4 to 8.

Items 1 and 2 as well as items 9 and 10 in table 3 again indicate that there is no cytoplasmic influence in this type of cross-sterility. These two sets of F_2 progenies from reciprocal single crosses show good agreement within single crosses as to percentages of non-receptive plants.

An opportunity to check the segregation of receptive to non-receptive plants in an F_2 progeny—(Hy × 845) F_2—as estimated from open-pollination in a detasselling plot against an estimate made in a population from the same seed by hand-pollinating the ears with *ga* pollen showed close agreement. In the hand-pollinated population we found 128 non-receptive plants, 9 doubtful, and 31 receptive plants for a percentage of non-receptive plants of 80.5. This compares with a percentage of 82.5 non-receptive plants in the detasselling plot where the segregation was 179 non-receptive, 7 doubtful, and 38 receptive plants (item 7, table 3). This would indicate that for this F_2 progeny the percentage of non-receptive plants as found in the detasselling plot is not biased by some plants which would normally show a normal phenotype receiving insufficient pollen and hence being classified as non-receptive.

DEFICIENCY OF SUGARY KERNELS IN F_2 PROGENIES

Many of the popcorn inbreds under consideration were used as pollinators on a sweet corn inbred, P51 (*ga su/ga su*), and on a line homozygous for the gametophyte factor and sugary, *Ga su/Ga su*. The respective crosses were *ga su/ga su* × *Ga*^{s} *Su/Ga*^{s} *Su* and *Ga su/Ga su* × *Ga*^{s} *Su/Ga*^{s} *Su*. The resultant proportions of sugary kernels in the F_2 progenies are of interest since most previous investigations of the gametophyte factors at this locus have been carried out by following the segregation of the *su* gene when linked either to *ga*, *Ga*, or *Ga*^{s}.

For four different cross-sterile popcorn inbreds plus line D139 (SCHWARTZ's *Ga*^{s}/*Ga*^{s}) table 4 gives the percentages of sugary kernels in the F_2's of crosses of these inbreds with P51 (*ga su/ga su*). Where the cross-sterile parent was D139—*Ga*^{s} *Su/Ga*^{s} *Su*—the percentage of sugary kernels was 12.3. This agrees well with the figure of 12.9 percent sugary which SCHWARTZ (1950) gives for the cross, *Ga*^{s} *Su/ga su* × *Ga*^{s} *Su/ga su*. When, however, the cross-sterile parent is any popcorn inbred, the percentage of sugary kernels is somewhat greater. Calculations of the significance of the differences of the means (of the percentage of sugary kernels on each ear) show that the means for each cross involving a popcorn inbred differ significantly from the mean of the cross involving D139. The difference between means is significant at the .05 level between D139 and 4524 and at the .01 level between D139 and 1001, D139 and 1491, and D139 and 24. That this is so for each of the popcorn

inbreds might indicate that they carry an allele Ga^{s_x} or alleles sufficiently similar to Ga^s that they give the same result in cross-pollination (*i.e.*, sterility to the dent inbred Hy), but different enough so that some *ga* gametes function in competition with Ga^{s_x} gametes on Ga^{s_x}/ga silks. In such cases, more sugary kernels would be expected in the F_2 progenies. Alternatively, more crossing-over between the Ga^s locus and the *Su* locus could also explain the observed results.

Also of interest are the percentages of sugary kernels in the F_2's of crosses of the various popcorn inbreds with *Ga su/Ga su*. These are given in table 4. Even larger disparities exist between inbreds than are found in crosses with *ga su/ga su*. The F_2 generation of (4524 × *Ga su/ Ga su*) shows 20 percent sugary kernels which is significantly less at the .05 level than 1491, and less at the .01 level than 1001, 24 and 845. Also SCHWARTZ (1950) found 23.4 percent sugary kernels in the F_2 of Ga^s *Su*/Ga^s *Su* × *Ga su/Ga su*. This figure

TABLE 4

Segregation of sugary kernels in the F_1's of crosses of P51 (ga su/ga su) and Ga su/Ga su times various cross-sterile popcorn inbreds and D139 (Ga^s Su/Ga^s Su), 1950.

Inbred	on *ga su/ga su*			× *Ga su/Ga su*		
	No. ears	Total kernels	Percent *sugary*	No. ears	Total kernels	Percent *sugary*
D139 (Ga^s)	13	4096	12.3	....	....	23.4†
1491-KKB	11	3585	15.5**	10	4012	22.6
1001-52	15	4558	15.5**	8	3133	23.1
4524-4	14	4859	13.9*	10	4623	20.0**
SA24	25	10004	14.4**	10	4146	25.2**
845-1B	....	....	....	7	2586	26.8**

†from SCHWARTZ'S (1950) data
* sign. different at .05 level from Ga^s
** sign. different at .01 level from Ga^s

which was derived from a large population probably represents a close approximation to the true percentage of sugary that should be obtained when *Ga* and Ga^s gametes are competing on Ga^s/*Ga* silks and *su* is linked to *Ga* with a recombination value of 25.3 percent. The percentages of sugary kernels in the F_2 of crosses of 4524, 24, and 845 times *Ga su* are significantly different on the .01 level from the 23.4 percent we might expect if the allele carried by these inbreds was actually Ga^s, and the same complement of modifying genes was present in all cases. Again a possible explanation of this divergence in percentages of sugary in the F_2 progenies of crosses × *Ga su/Ga su* is that the alleles carried by the popcorn inbreds are sufficiently different from Ga^s that they do not have the same competitive relationship to *Ga*, and in different cases either more or less *Ga* gametes may effect fertilization in competition with gametes carrying popcorn alleles than in competition with Ga^s gametes.

To summarize the foregoing data, four inbred lines of cross-sterile popcorn showing the same phenotype as Ga^s/Ga^s (*i.e.*, inability to set seed with pollen from *ga* lines) have significantly different percentages of sugary kernels in the F_2's of crosses with *Ga su/Ga su* and *ga su/ga su*. It is entirely possible when considering only one set of crosses (*e.g.*, times *Ga su/Ga su*) to hypothesize that the popcorn inbreds in question are carrying an allele or alleles much like Ga^s so that they react in a similar manner in cross-pollination tests but enough different to have altered competitive values in relation to *Ga*. A difficulty arises, however, when both sets of F_2 data are considered together in table 4. There appears to be a non-linearity of action of the alleles at the *Ga* locus for all the popcorn inbreds since for any inbred the percentage of sugary kernels in the F_2's of crosses with *ga su/ga su* is always greater than the percentage of sugary kernels in the F_2 of the cross of *ga su/ga su* times Ga^s *Su/*Ga^s *Su*. The percentage of sugary kernels in the F_2 of a cross of a popcorn inbred times *Ga su/Ga su*, however, may be significantly greater than, significantly less than, or about equal to (depending on the inbred) the percentage of sugary kernels in the F_2 of a cross of *Ga su/Ga su* × Ga^s *Su/*Ga^s *Su*. We had tentatively assumed that differences in the percentages of sugary kernels might be due to the existence in the popcorn inbreds of an allele or alleles at the Ga^s locus that might be less effective competitively than Ga^s. In light of these comparisons this would mean that in 4524 there is an allele less effective than Ga^s in competition with *ga* but more effective than Ga^s in competition with *Ga*; in 1001 and 1491 there are alleles that are less effective than Ga^s in competition with *ga* but equal to it in competition with *Ga*; and in 24 an allele less effective than Ga^s in competition with *ga* and also less effective in competition with *Ga*. The inbred, 24, to judge from this last instance carries an allele with no competitive advantage over *Ga* in spite of the fact that 24 is cross-sterile whereas *Ga/Ga* is not. Furthermore, for 845, another cross-sterile inbred, the percentage of sugary kernels in the F_2 of a cross with *Ga su/Ga su* is such (26.8) as to suggest a slight competitive advantage for *Ga* over the allele carried by 845. There are then, with respect to alleles at the Ga^s locus, two types of non-linearity of action—(1) The cross-sterile popcorn inbreds 24 and 845 are carrying alleles that render them cross-sterile to dent corn pollen, *ga*, yet in gametes these alleles have no competitive advantage over *Ga*, and *Ga/Ga* is not cross-sterile to *ga* pollen and (2) The cross-sterile inbred 4524 has an allele that is significantly less effective than Ga^s in gametic competition with *ga* and significantly more effective than Ga^s in competition with *Ga* to judge by the percentages of sugary kernels in the F_2's of the respective crosses. The apparent non-linear behavior of these alleles may well raise the question of the existence of a possible complex locus at Ga^s analogous to that recently shown by LAUGHNAN (1949) for the A^b locus in maize.

It has been suggested that a greater amount of crossing-over between the *su* locus and the *Ga* locus might account for the significantly larger percentage of sugary kernels found in the F_2's of crosses of *ga su/ga su* times the various

popcorn inbreds. This possibility would seem to be obviated by both sets of F_2 data for 4524. Here the percentage of sugary kernels in the F_2 of *ga su /ga su* times 4524 was significantly greater than in the F_2 of *ga su/ga su* times Ga^s *Su/*Ga^s *Su,* but the percentage in the F_2 of 4524 times *Ga su/Ga su* was significantly less than in the F_2 of *Ga su/Ga su* times Ga^s *Su/*Ga^s *Su* (as determined by SCHWARTZ). To explain these differences on the basis of altered crossover values would necessitate assuming greater crossing-over in one case and less crossing-over in the other which would seem to be unlikely.

The percentage of sugary kernels segregating in the F_2 of a cross of *ga su /ga su* times a popcorn inbred is of interest in one other connection. The Supergold inbred, 18, in cross-pollination tests gives the same results (inability to pollinate cross-sterile types) as the dent inbred, Hy, and might be assumed to be carrying the allele *ga*. In the F_2 population of the cross (18 times *ga su/ga su*) there was found to be a significant deficiency of sugary kernels. In a total of 6260 kernels 1453 or 23.21 percent were sugary. When tested for agreement with the expectation of 25 percent sugary kernels, the chi-square value is 10.687 showing $P < .01$. It is entirely possible that this deficiency of sugary kernels may be due to Sg18 carrying an allele ga_{1-18} such that gametes with ga_{1-18} are unable to fertilize Ga^s/Ga^s plants, but on silks of the constitution ga_{1-18}/ga are more effective competitively than *ga* gametes. This would explain the deficiency in the F_2 of sugary kernels.

In this connection, it is interesting to note that BRIEGER, TIDBURY and TSENG (1938) in the F_2 of a cross of Golden Bantam sweet corn times a red brittle line found that of a total of 8616 kernels only 22.8 percent were sugary. Just as with our case, both parental lines were assumed to be *ga/ga.*

Evidence of this sort shows that both for popcorn inbreds which have been assumed to be Ga^s/Ga^s and a popcorn assumed to be *ga/ga* there are differences in behavior from the type alleles. Whether these are due to the presence of other alleles of the same general type, as suggested, or due to different complements of modifying genes cannot yet be ascertained in most cases. For the purposes of discussion, we shall continue to assign to an inbred the type allelic designation based on cross-pollination reactions (*e.g.,* 24 as being Ga^s/Ga^s and 18 as being *ga/ga*).

THE EXTENT AND SIGNIFICANCE OF CROSS-STERILITY

Although reports of the gametophyte and cross-sterility factors at the *ga* locus date back a number of years (EMERSON 1924, MANGELSDORF and JONES 1926, and DEMEREC 1929), no attempt has previously been made to investigate the extent to which the cross-sterility or gametophyte factors might be present in cultivated varieties. As both MANGELSDORF and JONES and DEMEREC found, the gametophyte factors which they reported in popcorn (the source of SCHWARTZ's Ga^s is unknown), it is not unexpected that more instances of cross-sterility should be found in popcorn varieties and inbreds. It is surprising, however, to find that in the material we have surveyed cross-

sterility is the rule rather than the exception. Of the lines first tested (table 1), those from South American, Hulless, Black Beauty, Red, Ohio Yellow, Amber Pearl, and Yarling Yellow are cross-sterile, the line from White Rice cross-neutral, and the lines from Supergold and Baby Golden–1 normal. Since the first tests, lines from other varieties have been included, and we find that inbred lines from Strawberry, Baby Golden–2, and Tom Thumb are cross-sterile and a line from Early Yellow is normal. The two varieties designated as Baby Golden–1 and Baby Golden–2 above are distinctly different types of popcorn but both came to us under the name Baby Golden.

With some of the varieties listed above, only one inbred from that variety was tested. Where a large number of inbreds from a variety have been tested as with South American and Supergold, they have all proven to be of the same type within a variety (*i.e.*, all South American inbreds are cross-sterile and all Supergold inbreds are normal). It is worth noting that while the varieties giving rise to normal inbreds—Supergold, Baby Golden–1, and Early Yellow—seem to be rather similar types, the varieties from which cross-sterile inbreds are derived encompass a wide diversity of plant, ear, and seed characteristics. South American, Yarling Yellow and Ohio Yellow comprise a rather similar group of varieties that may have had a recent common derivation, but other than these each variety seems to be a distinct type.

In addition to the varieties mentioned above which we have been using in a plant breeding program, a number of South American accessions have been tested. We are indebted to DR. GRANER for seed of Mais " pisanckalla " from Cochabamba, Bolivia, and to DR. C. A. KRUG for seed of a number of Brazilian popcorn varieties for which we do not as yet have more than a code number. DR. EDGAR ANDERSON kindly sent us seed of an Asiatic popcorn for inclusion in our tests. That cross-sterility is again the most prevalent condition is shown in table 5. Each variety was pollinated by *ga* pollen and pollen from the variety was put on Ga^s/Ga^s plants. A test of this sort should

TABLE 5

Test pollinations of foreign accessions. 1949 and 1950.*

Variety	Source	Ability to set seed with *ga* pollen	Ability to pollinate Ga^s/Ga^s plants	Probable allele
Mais "pisanckalla"	Graner	–		Ga^s
Variedade 735	Krug	–	+	Ga^s
" 1004a	"	–	+	Ga^s
" 1060a	"	–	+	Ga^s
" 1060c	"	–	+	Ga^s
" 1061b	"	–	+	Ga^s
" 1063	"	–	.5	Ga^s
" 1064B	"	+	–	*ga*
" 1119	"	+	–	*ga*
Asiatic Popcorn	Anderson	+	–	*ga*

*A "–" indicates no seed set, a "+" full seed set, and a decimal a percentage of full seed set.

enable us to detect which of the three possible types of alleles at the *ga* locus the variety is carrying. If the popcorn is *ga/ga*, it will accept *ga* pollen but will not pollinate Ga^s/Ga^s plants. If it is Ga^s/Ga^s, it will not accept *ga* pollen but will pollinate Ga^s/Ga^s plants. If the variety is *Ga/Ga*, it will accept *ga* pollen and will pollinate Ga^s/Ga^s plants.

If the varieties that we have tested can be considered to be typical samples of commercial varieties in the United States and of varieties indigenous to Brazil, it seems that the majority of the popcorns are cross-sterile. Of the 24 varieties tested to date, 17 are cross-sterile (Ga^s), 1 is cross-neutral (*Ga*), and 6 are normal (*ga*).

To this point, we have tacitly assumed that all field corn varieties and inbreds are genetically *ga/ga*. It seems certain that no field corn inbreds are Ga^s/Ga^s since these inbreds would then be cross-sterile, and such a situation could not pass unnoticed in the extensive breeding programs devoted to corn. The same reasoning applies to sweet corn. However, if an inbred of either sweet or field corn were *Ga/Ga* the situation would be more difficult to detect since such an inbred would set seed readily with pollen from other inbreds which are *ga/ga*. But *Ga/Ga* inbreds when used as male parents would induce seed set in cross-sterile inbreds, and pollinating a cross-sterile line by any inbred known to be compatible with *ga* pollen constitutes a test for the presence of *Ga* in that inbred. To ascertain the possibility of any field corn line being able to fertilize cross-sterile popcorn lines and hence being *Ga/Ga* a number of tests have been made over the past four years using various cross-sterile lines as testers and pollinating them by a range of field corn inbreds. The results are summarized in table 6. In no case do we find any inbred capable of inducing seed set in any cross-sterile inbred. Therefore, no inbred tested is either *Ga/Ga* or Ga^s/Ga^s. Admittedly, the number of inbreds tested is small in relation to the number in existence, but an effort was made to take inbreds from diverse parentage—late Southern whites, early Northern yellows, and mid-season corn belt varieties. All gave a negative reaction as did the sweet corn inbred and the flint corn which were tested. It may well be that no field corn inbreds are capable of pollinating the cross-sterile popcorns. Limited tests with detasselled rows of cross-sterile types contiguous to dent corn nursery plots but isolated from popcorn pollen show that the plants in these rows have barren cobs.

To find that most popcorns are cross-sterile (Ga^s/Ga^s) while all field corns tested are *ga/ga* raises several questions of major importance to the hypotheses concerning the evolution of our modern varieties of maize. It is significant from an evolutionary standpoint when a group of varieties or a sub-species contains largely one allele at a locus whereas another group of varieties or another sub-species is apparently devoid of that allele (as here where most popcorns are Ga^s/Ga^s and apparently all field corns are *ga/ga*). It is doubly significant when one of these alleles constitutes an isolating barrier in one direction. It is not an absolute barrier but rather a conditional one since in nature a cross-sterile popcorn could be contaminated by a field corn

if some field corn plants first were pollinated by the popcorn. Then in the next generation the hybrid plants would be heterozgous, Ga^s/ga, and would produce one-half Ga^s gametes which could fertilize cross-sterile plants. Thus there would be an infusion of field corn germ plasm into the popcorn variety.

It is pertinent to the point under discussion to note that under conditions of open-pollination in a population where originally each plant was Ga^s/ga the theoretical expectation is that ½ of the ga alleles would be elimininated each generation, and the population would move rapidly towards fixation of the Ga^s allele. This follows naturally from the fact that Ga^s pollen-tubes have

TABLE 6

Test pollinations to detect the ability of various dent corn inbreds to pollinate cross-sterile inbreds and single crosses, 1947–1950.

Year	Inbred tested	Source	Cross-sterile females	Reaction of females*
1947	H21	Johnson County White	1499–51 (Hulless)	–
"	Tr	Troyer Dent (Reid)	1503–4 (Hulless)	–
"	H5	A Minn. selection	1499–L2 (Hulless)	–
"	C103	Lancaster Sure-Crop	1499–52 (Hulless)	–
"	Tr	Troyer Dent (Reid)	1502–1 (Hulless)	–
1948	Hy	Illinois High Yield	24–6 (South American)	–
1949	M14	(Br10 × R8) S	207 × 58 (SA), 1500–2 (Hulless), 1001–K (Hulless)	– – –
"	A	Funk Yellow Dent	"	– – –
"	WF9	Reid Yellow Dent	"	– – –
"	Os420	Osterland Dent	"	– – –
"	L317	Lancaster Sure-Crop	"	– – –
"	38–11	_____	"	– – –
"	187–2	Krug	"	– – –
"	33–16	Johnson County White	"	.2, – –
"	K41	Pride of Saline	"	– – –
"	I234	Iodent	"	– – –
"	R30	_____	"	.1, – –
"	Tr	Troyer Dent (Reid)	"	– – –
"	H5	A Minn. Selection	"	– – –
"	P51	Golden Bantam Sweet Corn	24–6 (South American) 1001–52 (Hulless)	– –
1950	A432	Kiowa Flint	1001–K (Hulless)	–

*A "–" sign means that the cross-sterile female did not set seed with pollen of the inbred in question.

a competitive advantage of almost one over the ga pollen-tubes in silks which are ga/Ga^s or Ga^s/Ga^s. We have directly tested on a small scale the ability of ga gametes to effect fertilization on Ga^s/ga silks when competing with Ga^s gametes. The F_2 progeny of a cross of Hy $(ga/ga) \times 845$ (Ga^s/Ga^s) were used individually as pollinators on Ga^s/Ga^s testers. If when the F_1 plant was selfed, any ga microspores were functional, there should be in the F_2 some plants which were ga/ga and thus unable to pollinate the Ga^s/Ga^s tester. This was not found. Of 153 plants in the F_2 all 153 induced full seed sets in the cross-sterile testers showing that no F_2 plant was ga/ga.

Evidence such as that just cited is useful not only in showing that the competitive behavior of *Ga*^{s} *vs. ga* gametes is as expected but also in understanding how an allele like *Ga*^{s}, which in the homozygous condition enforces for any plant so constituted a condition of limited cross-pollination in a naturally open-pollinated species can come to a state of fixation in a population. Fixation of *Ga*^{s} in a population would come in spite of the possible greater genetic homozygosity and concomitant selective disadvantage for such plants because of the almost complete selective advantage of gametes with *Ga*^{s} on *Ga*^{s}/*Ga*^{s} or *Ga*^{s}/*ga* silks. Once such a mutation as *Ga*^{s} is established in a population, it will proceed towards fixation.

The popcorns in general have been regarded as primitive types of maize, STURTEVANT (1894) and MANGELSDORF and REEVES (1939). MANGELSDORF and SMITH (1949) in material from a cave digging have illustrated convincingly the evolution of more advanced maize types from the very primitive type found in the lowest levels. This prototype is both a pod corn and a popcorn. There seems to be a high probability that the popcorns are progenitors of our more advanced maize types. Nothing in our data would tend to invalidate such a conclusion, but it would seem necessary to add the reservation that such an ancestral type or types could not have been among the cross-sterile varieties which seem to include the majority of the popcorns. This follows naturally from the fact that the *Ga*^{s} allele would spread so rapidly through any population into which it was introduced. Therefore, it does not seem possible to derive a population which is *ga/ga* from a population in which *Ga*^{s} is present.

Further discussions of the significance of cross-sterility as an aid in tracing the evolution of modern maize should be deferred pending a broader survey of its extent in nature. It would seem important that all the available South American varieties of popcorn, flint corn, and flour corn be tested to ascertain which allele at the *ga* locus they carry. Only then shall we be prepared to evaluate properly any possible evolutionary role of these alleles. However, our data seem to warrant the suggestion that the cross-sterile and normal popcorns may have had separate origins or diverged early in the course of their evolution.

THE MECHANISM OF CROSS-STERILITY

The possible mechanism of action of the cross-sterility factors has been a subject of speculation for all investigators interested in the gametophyte factors. MANGELSDORF and JONES (1926) postulated first that the deficiency of sugary kernels when a *Ga Su/ga su* plant was selfed was due to the differential rates of growth of *Ga* and *ga* pollen-tubes in *Ga/ga* silks.

DEMEREC (1929) working, as we now know, with a higher order allele, *Ga*^{s}, felt that his results ruled out the hypothesis of MANGELSDORF and JONES concerning the competitive disadvantage of the *ga* pollen-tubes with respect to the *Ga* pollen-tubes since in his work the popcorn strain used would not set seed with dent corn pollen. Here no competitive effect could be involved,

and the situation was felt to be " cross-sterility which might be due to a number of different causes, such as; inability of pollen with recessive gametophyte factor to germinate on the silks having dominant allelomorph; inability of recessive pollen-tubes to grow in silks with dominant allelomorph; or inability of recessive pollen-tubes to reach the ovules in plants having the dominant allelomorph." Nevertheless, DEMEREC's results with Ga^s cannot be considered to rule out the possibility that in (*Ga Su/ga su*) the deficiency of sugary kernels may be due to *ga* and *Ga* pollen tubes having such different growth rates on *Ga/ga* silks that *Ga* gametes almost always effect fertilization.

SCHWARTZ (1950) in considering DEMEREC's suggestions as to the possible bases of cross-sterility has shown that the first two (inability of *ga* pollen to germinate or to grow in Ga^s/Ga^s silks) are not applicable. He pollinated Ga^s/Ga^s plants with *ga* pollen and by fixing and staining the silks soon after pollination showed that the *ga* pollen both germinated on and grew into Ga^s/Ga^s silks. This is substantiated and extended by our observations. When Ga^s/Ga^s plants are pollinated by *ga* pollen, and other Ga^s/Ga^s plants are pollinated by Ga^s pollen, fixation at various intervals after pollination shows not only that both types of pollen germinate but also that germination is equally rapid in both types and that the growth rates of both are equal as far as they can be easily followed (into the vascular strands traversing the silks longitudinally).

SCHWARTZ has suggested another alternative—that failure to effect fertilization may be independent of stylar interaction and instead may be concerned with an abnormal reaction within the ovule such as an inability of the pollen tube to penetrate the embryo sac. It might also be suggested that fertilization takes place, but the zygote is overgrown by maternal tissue as is known to take place in certain incompatible pollinations. That neither one of these offers a satisfactory explanation can be inferred from two different sets of data. In the first place, when in a generally incompatible cross ($Ga^s/Ga^s \times ga$) some seed is set, as may happen under certain environmental conditions, this seed is always concentrated at the tip of the ear and not scattered over the entire ear as would be expected if either of the above hypotheses were valid. Secondly, if a Ga^s/Ga^s plant of a white endosperm type is pollinated by pollen from a yellow endosperm line which is *ga* and pollinated again 24 hours subsequent by its own pollen, the result is a full set of selfed seed (with the possible exception of a few crossed kernels at the tip of the ear). This effectively obviates the possibility that fertilization takes place followed by a collapse of the developing zygote. Further, it can be pointed out that the fact that *ga* pollen will fertilize *Ga/Ga* plants except when competing with *Ga* pollen cannot be explained on the basis of an abnormal reaction of any sort within the ovule.

MANGELSDORF and JONES (1926) in examining the F_2 seeds on ears from plants that were *ga De/Ga de* (where *de* is linked to *Ga* with a recombination percentage of 34.9 and *de/de/de* gives a defective endosperm type) found as would be expected in an F_2 population of this type that there was an ex-

cess of defective seeds but further that there was a higher percentage of defective seeds in the lower halves (33.0) than in the upper halves (30.6). This was interpreted as evidence favoring the hypothesis that *Ga* pollen-tubes have a faster growth rate than *ga* pollen-tubes on *Ga/Ga* silks and that the farther the pollen-tubes have to grow the more likely is the gamete with the faster growth rate to effect fertilization. EMERSON (1934) reported confusing results in attempts to determine whether differential pollen-tube growth might be involved in differential fertilization. BRIEGER (1937) has reported that for a second gametophyte character Ga_2/ga_2 on chromosome 5 the effect of the alleles upon pollen-tube growth was such that Ga_2 pollen-tubes grew faster than ga_2 pollen-tubes. We have taken advantage of our numerous F_2 populations from crosses of *ga su/ga su* × *Ga*^{s} *Su/Ga*^{s} *Su* to check the possibility of different percentages of sugary kernels in the upper and lower halves of the ears. The data are given in table 7, and five different F_2 populations are considered. In only one of the crosses, (P51 × 4524) F_2, does there seem to

TABLE 7

Segregation of sugary kernels in the upper and lower halves of self-pollinated ears of the F_1 crosses of P51 (ga su/ga su) times various popcorn inbreds and D139 (Ga^s Su/Ga^s Su), 1950.

Cross	No. ears	Upper halves		Lower halves		Percent sugary	
		Su	*su*	*Su*	*su*	Upper halves	Lower halves
(P51 + 24) F_2	13	2257	368	2448	382	14.02	13.50
(P51 × D139) F_2	11	1562	212	1692	248	11.95	12.78
(P51 × 1491) F_2	10	1461	275	1509	268	15.84	15.08
(P51 × 1001) F_2	13	1760	324	1771	333	15.55	15.83
(P51 × 4524) F_2	14	1988	348	2195	328	14.90	13.00
		9028	1527	9615	1559	14.48	13.95

be a significantly greater percentage of sugary kernels in the upper halves than in the lower halves while in two others, (P51 × 24) F_2 and (P51 × 1491) F_2, there are slightly higher percentages of sugary kernels in the upper halves of the ears.

In all the progenies referred to in table 7, there was an attempt to check a possible environmental influence on the competition, if such existed, between *Ga*^{s} *and ga* pollen-tubes by making part of the pollinations in the morning as early as practicable and the remainder in the afternoon. When the data are subdivided in this manner as well as into upper and lower halves for each ear, it is apparent that the excess of sugary kernels in the upper halves of the ears over the lower halves in the above three progenies is almost all attributable to pollinations made in the afternoon. This is illustrated in table 8 a and b for (P51 × 4524) F_2. It is significant that not only is there an excess of sugary kernels in the upper halves over the lower halves for pollinations made in the afternoon, but for every P.M. pollination there is an excess of sugary kernels in the upper half of the ear. In contrast to this, there

is no discernible trend in the A.M. pollinations. A similar grouping of data for (P51 × 24) F_2 gives for P.M. pollinations, upper halves 15.80 percent sugary kernels, lower halves 13.53 percent sugary and for A.M. pollinations upper halves 13.71 percent sugary, lower halves 13.69 percent sugary. With regard to (P51 × 1491) F_2, a summation shows P.M. pollinations, upper halves 15.79 percent sugary, lower halves 14.02 percent sugary and A.M. pollinations, upper halves 15.88 percent sugary, lower halves 15.85 percent sugary. The best explanation of these data would seem to be that in these three progenies under the different conditions prevailing in the afternoon, *ga* gametes were slightly but significantly more effective competitively than

TABLE 8

Segregation of sugary kernels in the upper and lower halves of self-pollinated ears of the F_1 cross (P51 × 4524) when pollinations were made at different times, 1950.

Ear	Upper halves		Lower halves		Percent sugary	
	Su	*su*	*Su*	*su*	Upper half	Lower half
			(a) P.M. Pollinations			
A	147	31	169	26	17.41	13.33
B	132	29	148	15	18.01	9.20
C	135	29	131	27	17.68	17.09
D	117	25	126	26	21.37	17.11
E	139	30	187	22	17.75	10.35
F	96	14	80	10	12.73	11.11
Total	766	158	841	126	17.10	13.03
			(b) A.M. Pollinations			
1	154	23	181	31	12.99	14.62
2	146	33	162	21	18.44	11.48
3	115	16	130	19	12.21	12.80
4	168	26	164	28	13.40	14.58
5	161	16	167	27	9.94	13.92
6	180	25	200	28	12.20	12.28
7	142	25	167	24	14.97	12.57
8	156	26	183	24	14.29	11.59
Total	1222	190	1354	202	13.46	12.98

they were in the morning. In contradistinction to these three progenies the other two (P51 × D139) F_2 and (P51 × 1001) F_2 show no hint of this excess of sugary kernels in upper halves of the ears over the lower halves in pollinations made in the afternoon. For (P51 × 1001) F_2 the figures are A.M. pollinations, upper halves 15.21 percent sugary, lower halves 15.77 percent and P.M. pollinations, upper halves 16.01 percent sugary, lower halves 15.95 percent sugary. For (P51 × D139) F_2 a summary gives for A.M. pollinations, upper halves 11.85 percent sugary, lower halves 13.02 percent and P.M. pollinations, upper halves 12.08 percent sugary, lower halves 12.50 percent sugary. From the above data showing that the detection of differential growth rates of pollen-tubes may depend not only on the lines involved but

also on environmental conditions, it is little wonder that previous investigators have obtained contradictory results in experiments designed to detect the presence of such a phenomenon. A further possible source of confusion is indicated by MANGELSDORF (1929) whose data seemed to show that on *ga/Ga* silks *ga* pollen tubes were slower growing than *Ga* pollen-tubes for most of the normal stylar length but might thereafter be more rapid.

In discussing the possible mechanisms of cross-sterility, several salient points should be reiterated; differential growth of pollen-tubes can be demonstrated in some crosses under certain conditions; when a cross-sterile variety is pollinated by *ga* pollen and there is any seed set, it is concentrated at the tip of the ear; pollination of a cross-sterile plant (Ga^s/Ga^s) by its own pollen 24 hours after pollination with *ga* pollen results in an almost complete set of selfed seed; and *ga* pollen on Ga^s/Ga^s silks germinates as rapidly and has as fast an initial growth as does Ga^s pollen. With these facts in mind, it is difficult to find any hypothesis more plausible than that of a reaction between the diploid style and haploid pollen-tube. With specific reference to the alleles involved, the reaction between Ga^s/Ga^s silks and *ga* pollen-tubes is such that rarely can *ga* gametes effect fertilization and then only with ovules at the tip of the ear. With *Ga/Ga* silks and *ga* pollen the reaction is less severe so that a full seed set is obtained, yet the rate of pollen-tube growth must be adversely affected since when in competition with *Ga* gametes they rarely effect fertilization. The same conditions apply when the silks are heterozygous; *ga* pollen will usually induce seed set in Ga^s/ga plants when applied alone but not when competing with Ga^s pollen; *ga* pollen is effective on *Ga/ga* plants if not competitive with *Ga* pollen. The fact that *ga* pollen starts to grow so rapidly on Ga^s/Ga^s silks where it is usually not destined to effect fertilization suggests the possibility of nutritive difficulties. In this case the growth rate of both types of pollen-tubes would be equal until the pollen reserves are exhausted. After this point, there may be little or no pollen-tube growth (*ga* on Ga^s/Ga^s) or a reduced rate of pollen-tube growth (*ga* on Ga^s/ga, *Ga/Ga* or *Ga/ga*). Another possibility is that of an immune reaction of the antigen-antibody type where the antibody must be formed in response to the antigen before the growth of the pollen-tube is slowed or halted altogether. In either case with *ga* on *Ga/Ga* the reaction is not severe enough to keep the *ga* pollen-tubes from reaching every ovule but sufficient to keep them from effective competition with *Ga* gametes when both are present.

GENETIC MODIFICATION OF A CROSS-STERILITY REACTION

We have already suggested that the various popcorns while acting in tests approximately as though they were *ga/ga* or Ga^s/Ga^s show sufficient differences to imply that they may carry slightly different alleles of these classes or different modifying genes. The Supergold inbreds act in cross-pollination tests as though they are *ga/ga*, yet in F_2 progenies of crosses with cross-sterile inbreds there are significantly greater proportions of individuals accepting *ga* pollen than when field corn inbreds are used. Among the cross-

sterile popcorn inbreds there are significant differences in the percentages of sugary kernels in the F_2 progenies of crosses with *Ga su/Ga su* or *ga su/ga su* and in the percentages of normal individuals (accepting *ga* pollen) in the F_2 progenies of crosses with *ga*. Further evidence of differences between cross-sterile inbreds can be shown by reference to the F_1 progenies of 4519 × 24 and 4519 × 845 where 4519 is a cross-neutral popcorn inbred acting as though it were *Ga/Ga*. When both progenies are tested in a detasselling plot for receptivity to *ga* pollen, quite different results are obtained as shown in table 9. Clearly the two samples have not been drawn from the same population. These results are positively correlated with those in table 1 which show that as an inbred 845 is more cross-sterile than is 24.

With regard to one case of differential reaction in our cross-pollination tests it can be shown to be highly probable that the inbreds are carrying the

TABLE 9

Two F_1 progenies of cross-sterile popcorn inbreds (24 and 845) times a cross-neutral popcorn inbred, 4519, scored for receptivity to ga pollen.

Reaction*	4519 × 24	4519 × 845
	No. of plants	No. of plants
+	63	1
.9	27	0
.8	13	6
.7	5	6
.6	1	7
.5	2	12
.4	0	21
.3	0	13
.2	0	30
.1	0	13
–	0	1

*A "+" indicates full seed set, a "–" no seed set, and a decimal that proportion of full seed set.

same allele with different complements of modifying genes rather than two different alleles. In 1949 a cross-sterile Hulless inbred, 1001 KKB, was used as a tester and pollinated by a number of field corn inbreds and a normal popcorn inbred, 1708. The line was completely sterile to all the field corn lines as shown in table 6 but perfectly fertile with 1708. This differentiates it from a sister inbred 1001–52 which is completely sterile with 1708. It also shows that 1708 is not identical with the field corn inbreds including our usual *ga* tester, Hy. This might have been inferred from table 1 which shows some difference between Hy, 18, and 1708 in their ability to pollinate cross-sterile inbreds but not as clearly as do the above data. In 1950, 33 plants of the backcross (Hy × 1708) × Hy were used to pollinate plants of the inbred 1001–KKB. If Hy and 1708 were carrying different alleles of the general type *ga* such that ga_{1-Hy} could not fertilize 1001–KKB while ga_{1-1708} could, in the backcross progeny there should be equal numbers of plants

which were ga_{1-Hy}/ga_{1-Hy} and could not fertilize 1001–KKB and ga_{1-Hy}/ga_{1-1708} which could fertilize 1001–KKB because ½ of the gametes would be ga_{1-1708}. The exception was not realized since no plant of the backcross progeny could induce seed set in plants of 1101–KKB. Obviously the ability of 1708 to fertilize 1001–KKB while Hy cannot is not due simply to their carrying different alleles of the *ga* type. It seems that they may be carrying the same alleles with different modifiers or possibly different alleles with different modifiers sufficient to mask the allelic differences. No analysis has been made of the genetic difference between 1001–52 and 1001–KKB permitting one to be sterile with 1708 and the other fertile.

CROSS-STERILITY IN PLANT BREEDING

The cross-sterility allele of the type Ga^s has definite applications both in seed production and for use in hybrids which are to be grown for a particular endosperm type. In popcorn the major commercial types have been three-way crosses of the type (Supergold × Supergold) × South American. Since South American inbreds are sterile with pollen from Supergold inbreds, the crosses must be made as indicated above. The Supergold inbreds are perfectly fertile with dent corn pollen which is always present to some degree since complete isolation from field corn cannot be obtained in the Corn Belt. The kernels from ovules fertilized by gametes from field corn cannot be separated from those fertilized by the desired popcorn parent, but when planted produce hybrid plants giving starchy ears, whose kernels will not pop and which must be separated from the popcorn ears before processing. The percentage of contamination in seed produced in poorly isolated fields has approached 25 percent, thus lowering the value of the crop produced from the seed by that amount in addition to the labor expended in the necessary separation of the contaminants. It is, however, proving to be a simple matter to transfer the allele for cross-sterility (Ga^s) to these Supergold inbreds which insures in a seed production field that the female plants will set seed only with pollen from the male rows (South American and hence Ga^s) and not with any dent pollen which should happen to drift into the field.

A further and potentially more important use would be to transfer this cross-sterility factor to the inbreds comprising those hybrids which are being grown for a particular endosperm characteristic (e.g., waxy, high amylose, etc). These endosperm characteristics are genetically recessive so that there are two opportunities for contamination—first in the crop being grown for seed, and secondly in the crop being grown for processing. Contamination of the seed crop is usually detectable, and the crossed (starchy) kernels can be sorted out by hand labor. However, this costly and tedious procedure could be eliminated if the inbreds used in seed production were Ga^s/Ga^s. In the crop being grown for processing where dent corn pollen drifts into the field and effects fertilization, it reduces the value of the crop proportionally to the extent of fertilization with that pollen since the contaminated kernels will all have starchy endosperms and be of no use for the special process for which

the particular type of corn was being grown. If the industrial procedure demands raw material of high purity, the crop may even be useless. If, however, the hybrid were Ga^s/Ga^s in constitution, it would set seed only with its own pollen and 100 percent of the kernels would be of the desired endosperm type. With the increased breeding of hybrids for special endosperm characteristics, and the difficulty of isolating production fields of such hybrids from others which are a source of contaminating pollen, it would seem well worthwhile to expend the little effort required to transfer Ga^s into all the inbreds being used in hybrids grown for endosperm constitution, thus achieving genetic isolation.

PERRY (1945) has previously suggested that sweet corn inbreds be made *Ga/Ga* so that the hybrids would also be *Ga/Ga*, and hence potentially contaminating pollen grains, *ga*, from adjacent dent corn fields are at a competitive disadvantage and rarely effect fertilization. This was proposed for the same reason set forth above—to insure that all of the final crop will be of the desired composition. Here again it would be more desirable to transfer Ga^s rather than *Ga* to these inbreds because its greater potentiality for cross-sterility insures that *ga* pollen will not effect fertilization even in the absence of Ga^s pollen. This may be important in hybrid seed production fields where the males are a few days too early or too late for the female. It is under just such conditions that most contamination is found.

SUMMARY

1. Many varieties of popcorn will not set seed when pollinated by any field corn or certain other varieties of popcorn. No difficulty is experienced in making the reciprocal cross. The genetic basic of this non-reciprocal cross-sterility is a multiple allelic series at the *ga* locus on the fourth chromosome such that Ga^s/Ga^s plants will not set seed with *ga* (field corn) pollen but will set seed with either *Ga* or Ga^s pollen. Plants which are *ga/ga* or *Ga/Ga* will set with *ga*, *Ga* or Ga^s pollen. When both *ga* and Ga^s pollen-tubes are competing in ga/Ga^s styles, only the Ga^s gametes effect fertilization.

2. Direct tests of the inheritance of cross-sterility in backcross and F_2 progenies show that in most backcross progenies the expected segregations (1 receptive plant to 1 non-receptive plant or all non-receptive plants depending on the way the backcross was made) are closely approximated. In F_2 progenies there are wide deviations from the expected ratio of 1 receptive plant to 1 non-receptive plant.

3. The percentages of sugary kernels in the F_2 progenies of crosses of *ga su/ga su* times the cross-sterile popcorns and *Ga su/Ga su* times the cross-sterile popcorns differ slightly but significantly from the percentages of sugary kernels in the F_2 progenies of *ga su/ga su* and *Ga su/Ga su* times D139 (SCHWARTZ's Ga^s/Ga^s). The existence in the popcorns of different alleles of the same general type as Ga^s is suggested as a possibility.

4. The cross-sterile allele (Ga^s) is wide-spread both in the popcorns of commercial importance in this country and in South American popcorns. The

cross-neutral allele (*Ga*) has been found only in White Rice popcorn. No field corn has yet been shown to be of any constitution but *ga/ga*. The evolutionary significance of this is briefly discussed.

5. Cross-sterility may be relative rather than absolute. Where this is so, environmental influences may be decisive in conditioning the degree of seed set. Genetic modification may also play a major role in cross-sterility reactions.

6. The mechanism of cross-sterility is believed to be a reaction between the diploid style and the haploid pollen-tube such that the growth of *ga* pollen-tubes in Ga^s/Ga^s silks is halted and in ga/Ga^s silks is slowed to the point where they rarely effect fertilization when competing with Ga^s pollen-tubes.

7. The usefulness of the cross-sterility allele, Ga^s, in seed production and in the production of special endosperm types for processing is pointed out.

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A TRANSMISSIBLE DICENTRIC CHROMOSOME

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DICENTRIC chromosomes have been observed in many types of material, particularly following X-ray treatment, but they are usually eliminated within a few cell generations. An apparent exception to this rule is maize endosperm, where dicentrics are still found many divisions following their inception; but here a breakage-fusion-bridge cycle is set up (McCLINTOCK 1943, 1944), so that the dicentric is broken at each cell division, with sister ends uniting to form new dicentric chromosomes. Secondary centromeres have been described in rye by KATTERMANN (1939), PRAKKEN and MÜNTZING (1942), and ÖSTERGREN and PRAKKEN (1946); and in maize by RHOADES and VILKOMERSON (1942). These are not true centromeres, however, but are active at a particular stage of meiosis only. PIZA (1941) thought that the chromosomes of *Tityus bahiensis* were dicentric, but RHOADES and KERR (1949) have shown that this organism has either diffuse centromeres or many localized centromeres. The same is true of *Luzula purpurea* (CASTRO, CÂMARA and MALHEIROS 1949), and of certain coccids (HUGHES-SCHRADER and RIS 1941).

A transmissible dicentric chromosome in common wheat, *Triticum aestivum* L. *emend.* Fiori et Paoletti, was mentioned by SEARS (1946) and described in some detail by SEARS and CÂMARA (1950). Further observations on the behavior of this chromosome will be presented here, along with brief accounts of the findings reported in our previous paper, which was in Spanish. The chromosome has a "primary" centromere which is submedian and apparently normal, and a subterminal "secondary" centromere which is weaker than the primary but is active in both meiosis and mitosis. Figure 1 shows the relative lengths of the components of the chromosome, which are estimated at 30:10:1 for the long arm, the intercentromeric region, and the short arm, respectively. This chromosome was derived from an isochromosome for the short arm of chromosome VII. It was first observed in a plant which carried both the isochromosome and the dicentric. The parent of this plant had a single isochromosome and no other chromosome VII or derivative. Possible methods of origin of the dicentric will be discussed later.

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THE UNPAIRED DICENTRIC AT MEIOSIS

At meiosis the two centromeres of the unpaired dicentric chromosome almost always oppose each other, attenuating the intercentromeric region and orienting this region perpendicular to the metaphase plate. Thus the behavior of the dicentric is typical of that of a bivalent, except that it ordinarily lies off the plate nearer the pole toward which the primary centromere is directed (figs. 2 and 3). Rarely, as in figure 4, the dicentric chromosome is found lying on the plate. During anaphase, the intercentromeric region usually becomes further attenuated (fig. 5), through poleward movement of the primary centromere. The secondary centromere makes little, if any, progress toward its pole. Usually the entire chromosome is included within the one telophase group. Occasionally, however, breakage occurs in the intercentromeric region, and monocentric chromosomes are formed.

In about one percent of first meiotic divisions, the dicentric chromosome behaves as a univalent, coming late onto the plate and dividing. When this occurs, a bridge involving the intercentromeric region is usually formed between the two daughter chromatids (figs. 6 and 7). In the one microsporocyte (fig. 9) where no bridge was formed following division of the dicentric at AI, it was clear that the two centromeres of each chromatid were directed toward the same pole.

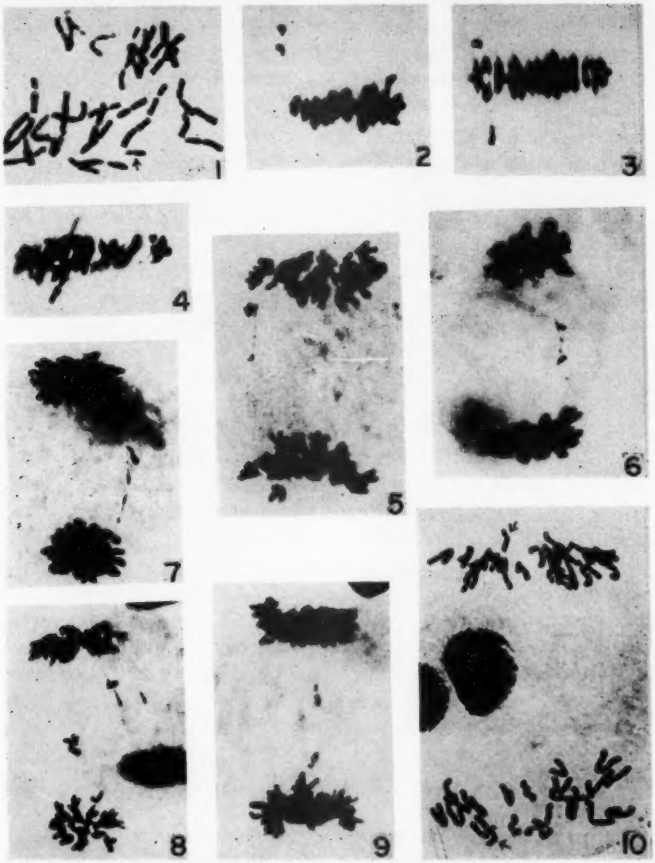
When it divides at the first division, the dicentric chromosome is subject to misdivision, like any ordinary univalent. The two misdivisions observed, one of which is shown in figure 8, were identical in character. The primary centromere misdivided, breaking one chromatid at that point. The long arm of this chromatid remained attached to the primary centromere of the other chromatid, while the intercentromeric region and minute arm formed a dicentric fragment.

When the dicentric has failed to divide at the first division, it divides at the second division. This division is usually normal, as in figure 10. Bridges have been observed only occasionally. Presumably at this stage the two centromeres of each chromatid are usually oriented toward the same pole, with no twists or half-twists of the chromatids about each other in the intercentromeric region. Where a half-twist occurs, the two centromeres of each chromatid proceed toward opposite poles, forming a two-strand bridge. Such bridges may eventually resolve themselves without breakage.

The much higher frequency of bridges following division of the dicentric at the first division may be attributed to the more tightly coiled condition of the chromosome at that stage, such that there is usually at least a half-twist in the region between the two centromeres.

PAIRING BEHAVIOR OF THE DICENTRIC

Several plants obtained had a pair of dicentric chromosomes. In 93 percent of 200 microsporocytes classified in these plants, the two dicentrics were paired with each other. Where they were unpaired (figs. 2 and 3), each showed the typical behavior already described—that is, with both centromeres



FIGURES 1–10.—The dicentric chromosome in mitosis and meiosis. From aceto-carmine smears. × 790. FIGURE 1.—Part of a mitotic metaphase plate from a root-tip smear. The dicentric chromosome is indicated by arrow. FIGURES 2, 3.—Characteristic behavior of unpaired dicentrics at MI. FIGURE 4.—Unpaired dicentric (second from right) on plate at MI. FIGURE 5.—Typical behavior of unpaired dicentric at AI, passing undivided toward upper pole. Intercentromeric region attenuated. FIGURES 6–9.—Unpaired dicentrics dividing at first division. Bridges are present in figures 6 and 7. In figure 8 the dicentric has misdivided at the primary centromere. A portion of one chromatid, consisting of the intercentromeric region and minute arm, lies at the right. This fragment is presumably dicentric. The long arm of the broken chromatid remains attached to the primary centromere of the other chromatid. In figure 9, the secondary centromere of the upper daughter chromatid is leading the way toward the pole. FIGURE 10.—Typical AII, with a daughter dicentric at each pole.

active. Where they were paired, only one centromere of each chromosome was active, as a rule. The typical bivalent had a chiasma between the long arms of the two dicentrics, with the short arms (intercentromeric region plus minute arm) lying free. The chiasma was either interstitial (fig. 11) or terminal (figs. 12 and 13). These bivalents could only be distinguished from other bivalents of the complement by their smaller size and more extreme arm ratio.

In some microsporocytes, chiasmata occurred on both sides of the primary centromere, so that ring bivalents were formed (fig. 14). Occasional bivalents were found with a chiasma only in the intercentromeric region or perhaps in the minute arm. It could not be determined whether or not the secondary centromere, as well as the primary, was active in these bivalents.

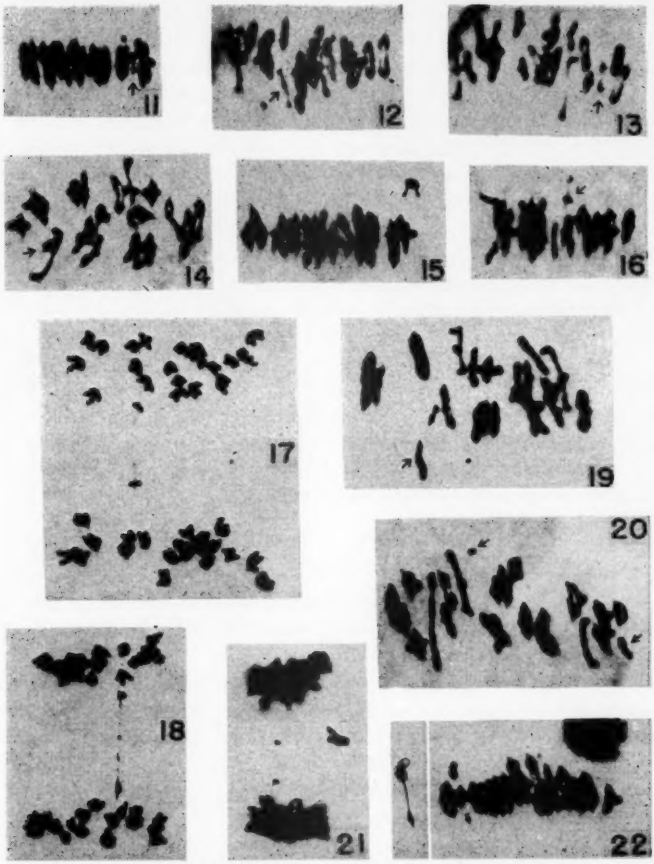
In another type of bivalent (fig. 15) both centromeres of each chromosome were active, but the two centromeres of each chromosome opposed each other rather than their homologues in the other chromosome. This resulted in metaphase behavior like that of two univalent dicentrics, but with the long arms of the two chromosomes joined. Presumably the two long arms had been paired in prophase throughout part of their length, while the centromere regions were involved in intrachromosomal pairing. Since both primary centromeres were oriented toward the same pole, the bivalent was displaced toward that pole.

Besides the symmetrical bivalents, there were a very few of asymmetrical type. In these bivalents (fig. 16) the primary centromere of one chromosome was the active one, while in the other chromosome it was the secondary which was active. Since the primary centromere of one chromosome was opposed by the secondary of the other, the bivalent lay off the plate toward the pole toward which the primary centromere was directed.

Following pairing of two dicentrics, bridges were seen at first telophase in 13 percent of 108 microsporocytes. These bridges, two of which are shown in figures 17 and 18, always involved the intercentromeric region. It could not be ascertained whether any of these bridges were due to activity of the secondary centromere.

The dicentric chromosome was also obtained in plants which had a normal chromosome VII. Here pairing occurred in only 59.5 percent of 168 microsporocytes, and interstitial chiasmata were not found. The long arm of the dicentric usually paired with the short arm of chromosome VII, as in figure 19. In these bivalents the primary centromere was active, the secondary inactive. One bivalent was found in which the secondary centromere of the dicentric was active, with the primary centromere presumably inactive, and the long arm of the dicentric lying free. Bridges were rarely seen at first telophase, only one definite bridge having been found in about 50 cells in which pairing had occurred. This bridge apparently involved the intercentromeric region of the dicentric chromosome.

As mentioned earlier, the dicentric chromosome frequently gives rise to derivative chromosomes which have lost the region containing the secondary centromere. When present with a dicentric, these derivatives were found



FIGURES 11–22.—First meiotic division. From aceto-carmine smears. × 790. FIGURES 11–16.—Pair of dicentrics at MI. In figures 11–14 the bivalents differ from the others only by their smaller size and, in 11–13, by shortness of the free arms. In figure 15 the two centromeres of each chromosome are opposing each other, attenuating the intercentromeric region to form the two vertical limbs of the configuration. The paired long arms are seen in optical cross section between the two limbs. Figure 16 shows an asymmetrical bivalent, in whose lower half the secondary centromere is the active one. FIGURES 17, 18.—Bridges at AI resulting from pairing of two dicentrics. The bridge in figure 17, and possibly that in 18, has already resolved itself. FIGURE 19.—Heteromorphic bivalent consisting of a dicentric chromosome and a normal chromosome VII. FIGURES 20–22.—Supernumerary heteromorphic pair consisting of a chromosome from *Aegilops umbellulata* and a telocentric for the short arm of this chromosome. The chromosomes are unpaired in figures 20 and 21, paired in figure 22. The heteromorphic bivalent in figure 22 is shown separately in the drawing to the left of the photograph.

paired with the dicentric in 87 percent of 300 microsporocytes. The large majority of the bivalents formed were held together by chiasmata in the long arm only. Interstitial as well as terminal chiasmata were found, as in pairs of two dicentrics. Bridges were observed in 4.05 percent of 222 first telophases. These bridges all involved the intercentromeric region.

When two derivative chromosomes were present, these were found paired in 85.5 percent of 200 microsporocytes. Again, pairing was mostly or all in the long arm, and chiasmata were interstitial as well as terminal. No bridges were found in 93 first telophases.

THE DICENTRIC AT MITOSIS

Few observations have been made of the behavior of the dicentric chromosome at mitosis. There is little reason to suspect, however, that it behaves much differently than at the second meiotic division, where it passes unchanged through the large majority of divisions. Certainly it is true that in plants carrying a dicentric the chromosome is found intact in most microsporocytes. Sectors do occur, however, in which the terminal region containing the secondary centromere has been lost; and bridges have been observed occasionally in telophases of root-tip mitoses.

TRANSMISSION OF THE DICENTRIC

Transmission data indicate that the dicentric chromosome, when unpaired, is less subject to loss than are normal univalents. On the female side, where no selection occurs, transmission was at least 41.7 percent (based on 60 offspring), whereas normal monosomes are transmitted through the egg to only 20 to 25 percent of offspring. This increased transmission is presumably due to the fact that the dicentric usually goes through meiosis like a bivalent, and hence is subject to loss at only the first division. As for male transmission, gametes carrying the dicentric are clearly favored over those lacking it, but no adequate data are available comparing its transmission with that of normal VII or with monocentric derivatives of the dicentric.

BEHAVIOR OF AN EXTREMELY HETEROMORPHIC BIVALENT

Two possible explanations were offered by SEARS and CÂMARA for the ability of the primary centromere to pull the unpaired dicentric off the plate in spite of the pull in the opposite direction by the secondary centromere. One explanation was that the primary centromere was intrinsically stronger than the secondary; while the other theory held that the primary was stronger by virtue of having a much larger portion of the chromosome under its control. If the latter explanation were correct, then heteromorphic bivalents, composed of one normal and one telocentric chromosome, should also lie off the metaphase plate, provided the disparity in size were great enough.

No chromosome in wheat is sufficiently heterobrachial to provide a critical test of this theory, but a chromosome has been added to wheat from *Aegilops umbellulata* (SEARS 1949) which has a marked disparity in arm length. Fol-

lowing misdivision of the unpaired Aegilops chromosome, a telocentric for the short arm was isolated and was then combined with the normal Aegilops chromosome. This provided a heteromorphic bivalent of which one member was at least six times as long as the other (figs. 20, 21)—approximately the same disparity as in the regions under the control of the two centromeres of the dicentric chromosome. Pairing of the telocentric with its normal homologue occurred in fewer than one percent of microsporocytes (3 in a sample of 460), but several good pairs were seen, of which some were definitely on the plate (fig. 22) and none was certainly off the plate. Therefore, it seems clear that the weakness of the secondary centromere cannot be attributed to its location, but must be considered an intrinsic characteristic.

DISCUSSION

SEARS and CÂMARA suggested two methods, neither of which is entirely satisfactory, by which the dicentric chromosome may have been derived from iso-VII. The first theory postulated the origin of the dicentric through an inversion involving one break within the centromere of iso-VII (fig. 23 A).

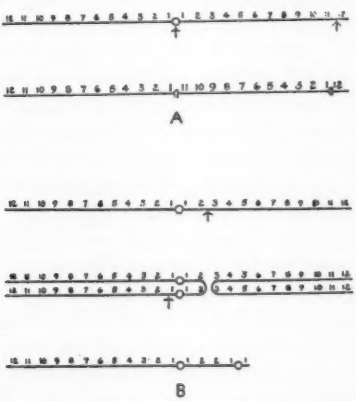


FIGURE 23.—Two possible methods of origin of a dicentric chromosome from an isochromosome: A. Through a simple inversion with one break in the centromere. B. Through a break, followed by union of sister ends, and then a second break.

This theory provides an explanation for the weakness of the secondary centromere, in that this centromere may consist of less than half of the original centromere. But the theory has the great weakness of not accounting for a chromosome with the proper relative lengths of its component parts. The long arm would be no longer than the intercentromeric region plus the short arm, whereas in both meiotic and mitotic divisions the long arm is seen actually to be about three times the length of the rest of the chromosome.

The second theory of the origin of the dicentric (fig. 23 B) has the isochromosome first losing most of one arm. Then the broken sister ends unite

to form a dicentric chromosome, and finally most of one of the free arms of this dicentric is lost. The resulting chromosome has, in order, one intact arm of the isochromosome; a centromere; a reversed duplication of the region adjacent to this centromere; a second duplication of this region, again reversed; a second centromere; and finally a smaller reversed duplication of the region adjacent to the second centromere. Thus one segment is duplicated three times, and a part of this segment is duplicated four times. An objection to the second theory is that it fails to explain the weakness of the secondary centromere. The theory may be modified so that it does explain the weak centromere, but before this is done, it will be well to compare the pairing behavior of the actual dicentric with that expected of the dicentric chromosome provided by the theory.

When present at meiosis as a monosome, the hypothetical dicentric shown in figure 23 B should frequently double back on itself to bring the two centromeres into opposition and thereby cause bivalent behavior. Often, however, the minute arm beyond the secondary centromere should pair with the identical region on the other side of this centromere, presumably preventing the secondary centromere from pairing with the primary, and thereby causing the dicentric to behave as a univalent. The fact that univalent behavior is seen in only about one percent of microsporocytes would seem to indicate either that the hypothetical chromosome does not correspond with the actual one, or that pairing of the minute arm tends to be suppressed.

Following loss of the region containing the secondary centromere of the hypothetical dicentric, the derivative monocentric chromosome would have homologous regions on the two sides of its centromere. Meiotic pairing and crossing-over between these regions would result in the two long arms of the divided univalent chromosome going to one pole and the two short arms to the other. In actual fact, such divisions are commonly seen. These figures resemble misdivision, but other types of misdivision figures, notably those with three arms going to one pole and one to the other, are not observed. Furthermore, in some figures one of the short arms can be seen disjoining from one of the long arms, as though the two had been paired.

When two hypothetical dicentrics are present, they should often pair with each other throughout their length and form a bivalent with all four centromeres active. That such bivalents occur rarely, if at all (those which obviously had all four centromeres active had not been paired with each other throughout their length), suggests that the two secondary centromeres are in some way prevented from pairing with each other. Intrachromosomal pairing of the minute arm with the region just across the secondary centromere might prevent these centromeres from pairing; but frequent occurrence of this type of intrachromosomal pairing is ruled out by the behavior of the univalent dicentric. The hypothetical dicentric provides a reasonable explanation of the asymmetrical bivalents observed, in that these could be the result of pairing of the intercentromeric region adjacent to the secondary centromere of one chromosome with the portion of the long arm of the other chromosome adjacent to the primary centromere.

It is in the pairing behavior with normal chromosome VII that the hypothetical dicentric is least satisfactory. According to the theory, the long arm of the dicentric is identical with the arm of chromosome VII (the short arm) which gave rise to the isochromosome. These two arms should therefore pair intimately. That they do not do so is indicated by the relatively low frequency of bivalents formed (about 60 percent) and by the absence of interstitial chiasmata in the bivalents that occur.

A modification of the theory could account for the weakness of the secondary centromere, by assuming a misdivision of the isochromosome. It is also possible to make the theory somewhat more plausible by (1) attributing the initial break to bridge formation following pairing between the two arms of the isochromosome, and (2) attributing the position of the second break to the tendency of a dicentric with one weak centromere to be displaced toward one pole. But detailed consideration of such refinements of the theory seems scarcely warranted when the final dicentric product has already been shown to be unsatisfactory. Development of an adequate explanation for the origin of the dicentric may not be possible until pachytene techniques are available for wheat which will reveal more fully the constitution of the dicentric.

Although the time of origin of the dicentric has not been definitely established, there is good reason to designate meiosis as the most probable occasion. At meiosis the parental isochromosome, which was present as a univalent, would have been undergoing misdivision with appreciable frequency. Under conditions leading to frequent misdivision, other abnormal processes would not have been unexpected. It is of course possible that the dicentric arose previous or subsequent to meiosis. If before meiosis, however, it could not have arisen long enough before to be present in all of one floret, for the zygote produced in the critical floret had the isochromosome as well as the dicentric. Neither could it have arisen long after fertilization, else a sector having the unchanged isochromosome should have been detected in the plant concerned.

When a dicentric is involved in pairing with another chromosome, a bridge should result at AI every time there is a chiasma in the intercentromeric region, provided that each chiasma represents a cross-over, as is generally believed. Double crossovers could occur without leading to bridges, but these should be rare in this short region. In plants with two dicentrics, chiasmata were actually seen at MI involving the intercentromeric region, in a frequency which, while not precisely determined, was of an order comparable to that of the frequency of anaphase bridges (13 percent) in the same plants. It was not certain that all of the bridges were due to crossovers, since if there were activity of all four centromeres in one bivalent, and if the two centromeres of each chromosome moved toward opposite poles, a bridge-like configuration might be the result at AI. The " bridge " in figure 17 may well be of this sort.

It has been assumed that the primary centromere of the dicentric is of normal strength and the secondary weak. From the mere fact that the primary outpulls the secondary when they are opposed, it might equally well be argued

that the secondary is normal and the primary abnormally strong. But from the behavior of pairs between normal chromosome VII and the dicentric, where the secondary centromere remains inactive, and of pairs between normal VII and derivatives lacking the secondary centromere, it seems clear that the primary centromere is of normal strength. These bivalents show no tendency to be displaced from the metaphase plate toward the pole to which the primary centromere of the dicentric is directed.

Since the secondary centromere is demonstrably weak, derivative chromosomes with only this centromere might be expected to show abnormalities of behavior such as lagging during mitosis. Where such derivatives have been observed at second telophase, however, they had divided and passed to the poles with no apparent tendency to lag. It must be concluded that a weak centromere shows its weakness only when opposed by a stronger centromere.

What is responsible for the weakness of the secondary centromere? Does it perhaps have a reduced amount of centromere substance? If so, this might mean deficiency for one of the two centromeric chromomeres observed by LIMA-DE-FARIA (1949) in the closely related genus Secale. The centromeres of telocentric chromosomes produced by misdivision are of normal strength, although these too presumably contain but a single centromeric chromomere; but possibly the terminal position permits more efficient functioning of the defective centromere.

If the secondary centromere of the dicentric chromosome is a deficient centromere, a ready explanation for its origin is at hand in the phenomenon of misdivision. The isochromosome from which the dicentric arose was present as a monosome, and hence subject to misdivision in every sporocyte. If weak centromeres arise in this way in appreciable frequency, monocentric chromosomes with weak centromeres might also appear following misdivision. None have yet been demonstrated, but scarcely any data are available on the centromere strength of dibrachial chromosomes issuing from misdivision.

SUMMARY

A dicentric chromosome in common wheat originated from an isochromosome for the short arm of chromosome VII. Its three parts have a length ratio of about 30 : 10 : 1.

At meiosis the dicentric usually behaves as a bivalent, with the two centromeres opposing each other. Since the sub-terminal centromere is weak, the chromosome lies off the plate at MI, and is commonly included entire in one telophase group.

At the second meiotic division and in mitosis the dicentric usually divides normally, with the two centromeres of each chromatid being directed to the same pole.

When the dicentric pairs with another chromosome, only one of its centromeres is active, as a rule, and this is almost always the primary centromere.

An extremely heteromorphic bivalent was not displaced from the metaphase plate. Therefore, the weakness of the secondary centromere is believed

an intrinsic property rather than being due to the position of this centromere in the chromosome.

Of two possible methods suggested for the origin of the dicentric from iso-VII, neither is entirely satisfactory.

The defectiveness of the secondary centromere seems likely to have resulted from misdivision of the isochromosome. The weakness of this centromere is only expressed when it is opposed by a stronger centromere.

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THE INFLUENCE OF HEAT TREATMENTS ON THE INJURY AND CYTOGENETIC EFFECTS OF X-RAYS ON BARLEY[1]

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IN favorable material, effects of ionizing radiations may be measured in terms of injury symptoms, cytological aberrations, and mutations. Usually these effects are associated with dosage, and increase or decrease together, *i.e.*, they are correlated. However, in earlier experiments in which it was shown that a heat treatment either before or after X-radiation modified the results of the irradiation, there was a suggestion that these criteria were not necessarily correlated. The present investigation was designed to test whether or not it is a fact that under certain conditions the injury, chromosomal aberrations, and mutations resulting from x-radiation may not be correlated. The data reported herein show conclusively that injury symptoms can be more or less divorced from genetic changes when X-rayed barley seeds are treated with heat either before or after the irradiation. Further, they show that a positive correlation does not necessarily exist between the mutation frequency and the frequency of chromosomal aberrations.

REVIEW OF LITERATURE

Various factors have been shown to modify the biological effects of X-radiation. This review deals only with reports on the influence of heat. The influence of this agency when applied before, during, and after X-radiation is discussed in that order. Studies on lower organisms are considered first, then animals, and lastly plants, whenever data on all three types of organisms have been found in the literature.

Relation of temperature to the biological effects of X-rays

Injury. There is little doubt that the temperature before, during, or after X-radiation can influence the injury resulting from the irradiation. However, the results are so affected by the experimental organisms and conditions that they are difficult to reconcile completely.

The writers have found no literature dealing with the effects of temperature before X-radiation on injury in lower organisms and animals. However,

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a number of such experiments have been conducted with plants. MAXWELL, KEMPTON and MOSLEY (1942) showed that temperature treatments of varying intervals from – 187°C to 56°C before X-radiation were ineffective in modifying X-ray-induced injury in dormant seeds of maize. In contrast with these results, SMITH (1943, 1946) found that pre-treating dormant seeds of wheat with heat decreased the sensitivity of the seeds to X-radiation.

The temperature during X-radiation is also important in modifying X-ray-induced injury. LEA, HAINES and COULSON, according to LEA (1947), found that the killing effect of X-radiation in bacteria was independent of the temperature during irradiation. However, TASCHER (1929) and STADLER (1931) observed that keeping dormant seeds of barley at – 68°C in "dry ice" during X-ray treatment reduced the injurious effect of the radiation as compared with the injury obtained at room temperature. Approximately a two-thirds larger dose of X-rays could be tolerated. TASCHER (1929) also found that a slight increase in injury resulted from exposure of the seeds to a temperature of 60°C during X-ray treatment. In another test he irradiated germinating barley seeds at 10°, 24°, and 35°C. Those at 24°C during the X-radiation were injured least and those at 10°C were injured most. MAXWELL and KEMPTON (1939) reported that when seeds of maize were X-rayed at the temperature of liquid air, the extent of plant growth before death was greater than when they were irradiated at room temperature. KEMPTON and MAXWELL (1941) also showed that dormant seeds of maize held at – 187°C were much less sensitive to irradiation than were seeds held at room temperature, and that seeds kept at 61°C during irradiation produced the best and largest seedlings. In another experiment seeds were X-rayed at – 187°C, – 66°C, 0°C, 27°C, and 56°C. Seeds at 0°C and 27°C were injured more than were seeds maintained either above or below those temperatures.

Apparently the temperature subsequent to irradiation is also important. TASCHER (1929) reported that irradiated dormant seeds of barley stored for seven weeks, and irradiated germinating seeds stored for 24 hours at 6°C, 22°C, and 38°C survived best at the lowest temperature. MAXWELL, KEMPTON and MOSLEY (1942) showed that post-treatment of X-rayed maize seeds with various temperatures between 56°C and – 187°C was ineffective in altering X-ray-induced injury. On the other hand, Smith (1943, 1946) reported that a heat treatment applied to wheat seeds following X-radiation increased the injury. SMITH and CALDECOTT (1948) indicated that such a treatment was ineffective in altering the survival to maturity of barley seeds.

Chromosomal aberrations. Experiments with grasshopper neuroblasts and dormant barley seeds indicate that the temperature before X-radiation can modify the frequency of X-ray-induced chromosomal aberrations. GAULDEN (1947, 1949) showed that treatment of grasshopper neuroblasts at 47°C immediately before X-radiation significantly reduced the frequency of double fragments, and caused an increase in the number of single fragments when compared with pre-treatment at 43°C. SMITH and CALDECOTT (1948) reported that the frequency of chromatinic bridges in root-tip cells of barley was

reduced by half when dormant seeds were subjected to a barely sub-lethal heat treatment before X-radiation.

The temperature during irradiation is apparently important in both plants and animals in modifying the frequency of X-ray-induced chromosomal aberrations. MULLER (1930), from his work with Drosophila, was one of the first to admit the possibility that temperature could affect the frequency of chromosomal aberrations induced by X-radiation. PAPALASHWILI (1935), MICKEY (1939), and KING (1947) reported that X-radiation of fruit flies at low temperatures (approximately 0°C) produced a higher frequency of chromosomal aberrations than was produced at room temperature. MICKEY noted that the frequency of interchanges was about twice as great at the lower temperature. STADLER (1930) suggested the possibility that the temperature during X-radiation may modify the production of X-ray-induced chromosomal aberrations in plants. SAX and ENZMANN (1939) found that low temperatures during X-radiation increased the frequencies of chromosomal aberrations produced at mid-prophase, or the resting stage in microspores of Tradescantia. The temperature effect was reversed when irradiation was applied at earliest prophase when the chromosomes were split to form sister chromatids. CATCHESIDE *et al.* (1946), also working with Tradescantia microspores, noted that the yield of X-ray-induced chromatid breaks was only slightly dependent on temperatures from 1° to 30°C during the irradiation. The yield of interchanges produced by a given dose of X-rays also diminished with an increase in exposure time at 1° and 20°C. FABERGÉ (1940), using the same test material, found that X-radiation at a temperature of 30°C produced fewer fragments than did the same dose at 15°C. SAX (1947, 1950) observed that the frequencies of ring and dicentric chromosomes were several times higher in Tradescantia microspores X-rayed at 3°C than at 36°C. FABERGÉ (1948, 1950) found only one-fifth as many aberrations in divisions of generative nuclei of Tradescantia microspores X-rayed at the temperature of liquid air as compared with the frequency in those which were irradiated at room temperature.

Experiments also have been conducted to determine whether or not different temperatures after X-radiation can modify the frequency of X-ray-induced aberrations. GAULDEN (1949) found that treating grasshopper neuroblasts with 43° or 47°C immediately after X-radiation caused an increase in the total number of breaks induced by the X-rays. On the other hand, SAX and ENZMANN (1939) claimed that an increase in the frequencies of chromosomal aberrations produced at mid-prophase, or the resting stage in microspores, could be effected by using low temperatures immediately after X-radiation. The effect was reversed when the chromosomes were irradiated at early prophase. SMITH and CALDECOTT (1948) showed that the frequency of X-ray-induced chromatinic bridges was reduced by half when dormant seeds of barley were subjected to a barely sub-lethal heat treatment immediately after X-radiation as compared with seeds X-rayed at room temperature.

Mutations. Opinions differ as to whether or not the frequency of X-ray-induced mutations can be modified by temperature treatments before, during, or after X-radiation. The writers have found no literature pertaining to animals which deals with the effects of temperatures before X-radiation on the mutation frequency. However, SMITH and CALDECOTT (1948), working with dormant seeds of barley, obtained a mutation frequency of 5.8% from X-radiation alone and 6.9% when the seeds were subjected to a barely sub-lethal heat treatment immediately before X-radiation. This difference was not significant.

The effect of temperature during X-radiation on mutation frequency has been studied by a number of workers. MULLER (1930), TIMOFÉEFF-RESSOVSKY (1934), and MAKHIJANI (1944), were unable to detect any relation between X-ray-induced mutation frequencies and the temperature at which Drosophila were irradiated. On the other hand, MEDVEDEV (1935), KING (1947), and NOVITSKI (1949) found higher mutation frequencies in flies irradiated at low temperatures (approximating 0°C) than in flies irradiated at room temperature. NOVITSKI observed an increased mutation frequency of 75 percent per roentgen unit when the flies were irradiated at – 5°C as compared with room temperature. BAKER and SGOURAKIS (1950) obtained similar results. They noted a significantly greater frequency of lethal mutations in male flies X-rayed in oxygen at 2°C as compared with 27°C. The temperature effect was not apparent when the flies were irradiated in nitrogen. STADLER (1928b) found no clear difference in the mutation frequency of either dormant or germinating seeds of barley given equivalent doses of X-rays at 10°, 20°, 30°, 40°, and 50°C, although he (1930) admitted the possibility of such an effect. However, he (1931) found that the mutation frequency was decreased per "r" unit if dormant seeds of barley were X-rayed in dry ice as compared with a "normal" temperature.

There is evidence that the temperature to which organisms are subjected after X-radiation may also affect the mutation frequency. NOVITSKI (1949) reported that the frequency of X-ray-induced mutations in *Drosophila melanogaster* could be increased by decreasing the temperature immediately after irradiation. SMITH and CALDECOTT (1948), working with dormant seeds of barley, claimed that the frequency of X-ray-induced mutations was increased from 5.8 percent in seeds subjected to X-radiation only, to 9.1 percent in seeds X-rayed and then immediately subjected to a sub-lethal heat treatment.

There is also some evidence that X-radiation can modify the effects, primarily injury, produced by high temperatures. According to GIESE and HEATH (1948), *Paramecium caudatum* is sensitized to heat by a prior X-ray treatment. CALDECOTT and SMITH (1948), and NYBOM (1950), on the other hand, reported that X-radiation could give a measure of protection from a subsequent heat treatment. However, this result was not obtained by SUSKIND (1950).

MATERIAL AND METHODS

Barley seeds used

Dormant seeds of diploid barley, variety Himalaya (C.I. 620), were used in these experiments. Tests run in 1947 were performed with the stock orginally obtained from D. W. ROBERTSON. In order to obtain as nearly homozygous a line as possible for future studies, a single plant selection was increased in the summer of 1948. Since 1949 the tests have been made with the progeny from this single plant.

Treatments

In all experiments the following treatments and combinations of treatments were given: 1) X-radiation only, 2) heat only, 3) X-radiation immediately followed by heat, 4) heat immediately followed by X-radiation. The dosages of X-rays and heat used in the various treatments were determined on the basis of preliminary trials not reported here. The moisture content of the seeds was not determined before the experiments were conducted. However, moisture was not a variable between treatments of a given experiment, but may have changed slightly between experiments run several months apart. For the experiments conducted in 1950, all seeds used were stored in a humidity-controlled desiccator for at least six weeks before treatment.

X-radiation was applied to the seeds as they lay in a single layer on a piece of heavy blotting paper. In 1947 the X-ray apparatus was operated to give approximately 1,000r per minute at the surface of the seeds. In 1949 and 1950 the rate was reduced to 500r per minute, in a specially designed lead box. The X-ray treatments in 1947 were applied at 26.5 KVP and 25 ma. In 1949 and 1950 treatments were applied at 34 KVP and 27 ma. Two kenotrons gave full-wave rectification, and voltage variations were smoothed out by a capacitance of 0.18 microfarads to ground. Voltages were read on a microammeter in series with a high resistor.

For the heat treatments, seeds were placed on a 4×6-inch piece of blotting paper in a thermostatically controlled electric oven. For most treatments, the temperature did not vary more than 2°C. The heat treatments were applied at the highest temperature which permitted the survival of essentially all of the treated seeds. This temperature varied somewhat for the different experiments, possibly because of differences in the moisture content of the seed. In almost every test, if any of the seeds survived, practically all of them did. The temperature used for each experiment is given in the tables.

Growing conditions for treated seed

Conditions of germination and growth depended upon the information desired. 1) When grown for data on seedling emergence and height, the seeds either were planted directly in greenhouse flats or germinated in petri dishes. In the latter case they were covered with sand and grown in a daylight germinator. 2) When grown for data on root-tip aberrations and seed-

ling growth, seeds were germinated in petri dishes. After one to three root tips were removed from each seed, they were covered with sand and grown in a daylight germinator for seedling observations. 3) When grown for data on the frequency of chimeras, interchanges, and mutations, the treated seeds were planted in the field. Spikes for cytological analysis were removed at random from each treatment about two weeks before heading. No more than one spike was removed from any one plant. Notes on chimeras were taken at about the meiotic stage. At maturity the spikes were harvested from each plant separately and grown in (X_2) head progeny rows in greenhouse benches to determine the mutation frequency (after STADLER 1930).

Methods used in cytological studies

For cytological observations the acetocarmine smear technic (SMITH 1947) was used throughout. Carnoy's solution (6 parts 95% ethyl alcohol : 3 parts chloroform : 1 part glacial acetic acid) was used as a fixative. Root-tip cells at late anaphase were examined for chromatinic bridges. The frequency of interchanges was determined from observations on the first meiotic division in microsporocytes.

It was impracticable to obtain cytological observations on all plants grown for seedling observation or mutation frequencies. For this reason, the practice was adopted of taking random samples of the treated material for cytological data. However, an effort was made to obtain root-tip observations on the same experiments run for the frequency of interchanges and mutations. Unfortunately, for some reason no divisions were found in the root tips taken from the test run in 1949.

EXPERIMENTAL RESULTS

Injury symptoms

Survival. Survival was one criterion used for measuring the injury resulting from the heat and X-ray treatments. The writers (unpublished observations) and a number of other workers have observed that, particularly at higher dosages of X-rays, there is a delayed killing. Thus, if survival is measured by the number of seedlings which emerge or grow to the one- or two-leaf stage, a higher survival value is obtained than if the information is determined from plants which reach maturity. For this reason, survival data were based on mature plants. Delayed killing was not a characteristic response to heat treatments, so seeds which germinated survived about as well as untreated seeds.

Table 1 presents data on the effects of X-rays, heat and combination treatments on survival. It is apparent without statistical analysis that there was no important difference in the survival of plants from seeds given X-radiation alone or in combination with a pre- or post-treatment with heat.

Seedling height. Seedling height was also used for measuring injury. These measurements were made on the seedlings about 14 days after treatment. At this stage the first leaf had reached maximum growth and the

TABLE 1

Effect of X-rays (15,000r), heat, and combination treatments as measured by survival to maturity (summary of three tests).

Treatment	1947[1]		1949		1950		Total seeds treated	Weighted average survival
	Seeds treated	Survival	Seeds treated	Survival	Seeds treated	Survival		
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Control	....	....	450	94	400	90	850	92
X-rays	2950	29	950	45	1000	46	4900	37
Heat[2]	820	61	450	91	400	89	1670	76
X-rays plus heat[2]	2950	31	950	42	1000	50	4900	37
Heat[2] plus X-rays	2950	20	950	47	1000	47	4900	30

[1] In 1947 severe soil washing and baking materially reduced survival.
[2] 75° C for 30 minutes in 1947; 80° C for 30 minutes in 1949; 85° C for 30 minutes in 1950.

second leaf was emerging or partly out, but there had not been much opportunity for recovery. Eleven such experiments were run, including a random sample of seeds from the treatments which were planted in the field. The data from these eleven experiments were summarized for presentation in table 2. According to " t " tests, all differences between means for the X-ray and combination treatments were significant at the 5 percent level. The difference between the mean height of seedlings given X-radiation alone and those given heat plus X-rays was significant at the 1 percent level. The height of the seedlings from seeds given X-rays plus heat was more variable from test to test than in other lots. In some experiments there was less and in others more injury than in the X-rayed-only series.

Cytogenetic effects

Frequencies of chromatinic bridges in root tips from treated seeds. Chromatinic bridges in cells of root tips grown from treated seeds were one

TABLE 2

Effect of X-rays (15,000r), heat, and combination treatments as measured by germination and seedling height (summary of eleven tests).

Treatment	Treated seeds planted	Seeds which germinated	Height at 14 days	
			Weighted mean	
	No.	*No.*	*cm*	*S.E.*
1. Control	600	596	13.6	0.35
2. X-rays	775	751	7.2	0.46
3. Heat[1]	775	765	13.4	0.27
4. X-rays plus heat[1]	775	746	8.0	0.67
5. Heat[1] plus X-rays	775	751	9.4	0.43

[1] 75° C to 85° C for 30 minutes. For individual treatments the highest temperature at which almost all of the seeds survived was used.

criterion used for measuring chromosomal aberrations. The frequency of chromatinic bridges was determined in seven separate experiments in which seeds received X-ray, heat, and combination treatments. X-ray dosages of 10,000r, 12,000r, 15,000r, and 20,000r were applied in different tests. All analyses showed that the bridge frequency was reduced by pre- or post-treatment with heat. Data from three separate experiments in which the X-ray dosage was 15,000r are summarized in table 3. In these three tests it is apparent that the bridge frequency was reduced by about one-half by pre- or post-treatment with heat. According to "t" tests the differences between the bridge frequency in seeds treated with X-rays only, and in those given a pre- or post-treatment with heat, were significant at the 1 percent level. It made little difference whether the heat treatment preceded or followed the irradiation. However, in most cases the bridge frequency was slightly less in the lots receiving the heat treatment before rather than after the X-radiation. Figures 1 to 4 illustrate the types of cells tabulated for frequencies of chromatinic bridges.

TABLE 3

Effect of X-rays (15,000r), heat, and combination treatments as measured by the frequency of chromatinic bridges in root tip cells (summary of three tests).

Treatment	Cells examined	Bridges observed		
		Total	Per cell	
	No.	*No.*	*Av.*	*S.E.*
1. Control	469	0	0	....
2. X-rays	364	215	.59	.02
3. Heat[1]	296	8	.03	.009
4. X-rays plus heat[1]	445	143	.32	.02
5. Heat[1] plus X-rays	393	112	.28	.02

[1] 75° C for 30 minutes in 1947, and 85° C for 30 minutes in 1950.

Frequency of interchanges in pollen mother cells of X_1 plants. Heterozygous interchanges at the first meiotic division were also used for measuring frequencies of chromosomal aberrations. These analyses are major operations in terms of time and space required, so data were obtained for only 1949 and 1950 tests. The results are presented in table 4. A heat treatment either before or after the X-radiation reduced the frequency of interchanges by one-third to nearly one-half. The reductions were significant at the 5 percent level. The effect of the combination treatments on interchange frequency was in the same direction and almost to the same degree as was the effect on the frequency of chromatinic bridges (cf. table 3). Figures 5 to 8 illustrate some of the configurations observed on which the frequencies of interchanges were determined.

Frequencies of seedling mutations in X_2 progenies. Frequencies of seedling mutants in X_2 progeny tests was still another method by which the effects of X-rays, heat, and combination treatments were measured. Since there was little difference between the results obtained in 1947, 1949, and 1950, the

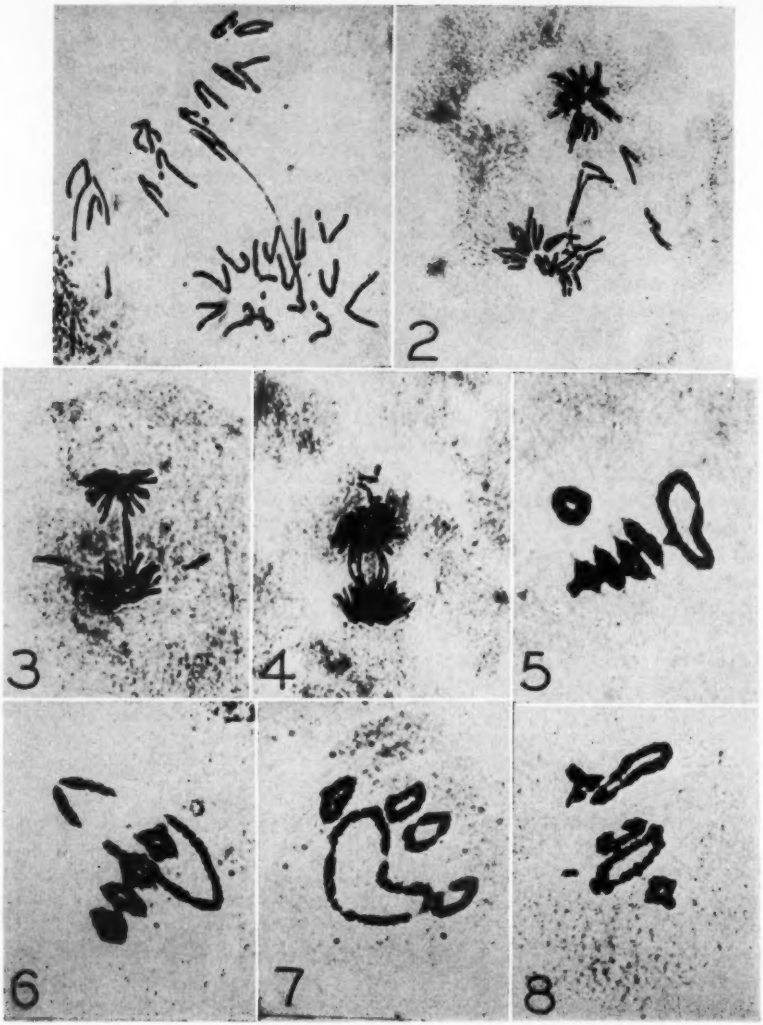

PLATE I

FIGURES 1 to 4.—Chromatinic bridges at anaphase of somatic mitosis. 1, one bridge; 2, 3, two bridges; 4, four bridges. × 1000.

FIGURES 5 to 8.—Interchanges observed at first meiotic metaphase. 5, ring-of-four and five bivalents; 6, 7, ring-of-six and four bivalents; 8, two rings-of-four and three bivalents. × 1000.

TABLE 4

Effect of X-rays (15,000r), heat, and combination treatments as measured by the frequency of interchanges at meiosis in X_1 spikes (summary of two tests).

Treatment	Spikes examined	Interchanges observed[1]		
		Total	Per spike	
	No.	*No.*	*Av.*	*S.E.*
1. Control	102	0	0	....
2. X-rays	258	72	0.28	0.03
3. Heat[2]	102	0	0	....
4. X-rays plus heat[2]	258	42.5	0.16	0.02
5. Heat[2] plus X-rays	258	50.5	0.19	0.02

[1] Calculated on a "ring-of-four" basis. That is, a ring-of-six was considered as 1.5 rings-of-four, involving a minimum number of breaks which could result in a ring-of-six.

[2] 80° C for 30 minutes in 1949, and 85° C for 30 minutes in 1950.

data were combined for presentation in table 5. A heat treatment either before or after X-radiation increased the mutation frequency over that produced in seeds by X-rays alone. These differences were significant at the 5 percent level. Thus, the heat treatments increased the frequency of mutations while at the same time decreasing chromosomal aberrations produced by X-radiation—a result which will be considered in more detail in the Discussion.

Frequencies of different types of mutants. Since a heat treatment either before or after X-radiation increased the mutation frequency, it was of interest to determine whether there was a proportional increase in all types of mutants or a disproportionate increase in some one or a few particular types. Observations on the types of mutants resulting from the different kinds of treatments are summarized in table 6. The chi-square values confirm the impression already gained from going over the material that there was probably no difference in the relative proportions of the mutant types resulting from the three combinations of treatment. The chi-square values for "yellow" and "shrivel-tip" approach significance, suggesting that there may

TABLE 5

Effect of X-rays (15,000r), heat, and combination treatments as measured by the frequency of seedling mutants in X_2 progenies (summary of three tests).

Treatment	Head progenies tested	Mutants obtained	Heads with mutants	
	No.	*No.*	*%*	*S.E.*
1. Control	911	2	0.2	....
2. X-rays	6283	359	5.7	0.01
3. Heat[1]	2151	4	0.2	....
4. X-rays plus heat[1]	5994	542	9.0	0.01
5. Heat[1] plus X-rays	6652	515	7.7	0.01

[1] 75° C for 30 minutes in 1947; 80° C for 30 minutes in 1949; 85° C for 30 minutes in 1950.

TABLE 6

A comparison of the relative frequencies of various types of mutants produced by X-rays (15,000r), heat, and combination treatments (summary of three tests).

Mutant types	Treatment						Total		
	X-ray		X-ray plus heat[1]		Heat[1] plus X-ray				
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*	χ^2
White	161	44.8	232	42.8	228	44.3	621	43.8	0.2404
Yellow	64	17.8	84	15.5	61	11.8	209	14.8	5.4435
Yellow green	63	17.5	92	17.0	100	19.4	255	18.0	0.9358
Virescent	17	4.7	24	5.0	27	5.2	71	5.0	0.1128
Striped (yellow, white)	15	4.2	26	4.8	17	3.3	58	4.1	1.4531
Banded shrivel	8	2.2	18	3.3	16	3.1	42	3.0	0.8939
Shrivel tip	10	2.8	18	3.3	7	1.4	35	2.5	4.3602
Others	21	5.8	45	8.3	59	11.4	125	8.8	7.7811
Total	359		542		515		1416		21.2208

[1] 75° C for 30 minutes in 1947; 80° C for 30 minutes in 1949; 85° C for 30 minutes in 1950.

be an effect of treatment on these types. The chi-square value for the group of mutants classed as "others" was large enough to indicate significant differences between treatments. However, the writers are of the opinion that this difference should not be accepted as reliable because of the small size of the population, classification difficulties, and the diversity of mutant types observed. It is probably more remarkable that the wide range of mutants obtained was so uniformly classifiable as to types.

Frequencies of chimeras in X_1 plants. A number of workers have reported finding occasional radiation-induced chimeras. RANDOLPH (1950) is the only worker known to us who has used chimeras as a measure of induced effects. Since these chimeras were rather common in our material, an attempt was made to determine their frequency (table 7). A dose of 15,000r units produced a chimera in about 2½ percent of the plants grown from the irradiated

TABLE 7

Effect of X-rays (15,000r), heat, and combination treatments as measured by the frequencies of chimeras observed in X_1 plants (summary of two tests).

Treatment	Plants examined	Chimeras observed	Chimeras per plant
	No.	*No.*	*Av.*
1. Control	785	0	0
2. X-rays	893	21	0.024
3. Heat[1]	767	0	0
4. X-rays plus heat[1]	899	24	0.027
5. Heat[1] plus X-rays	917	27	0.029

[1] 80° C for 30 minutes in 1949; 85° C for 30 minutes in 1950.

seeds. The heat treatments either before or after the irradiation seemed to have little effect on their frequency. The chimeras found were commonly either yellowish or white stripes, and in most cases were first detected between the three-leaf and the flag-leaf period of growth. Most of the chimeras became progressively more difficult to detect as the plants approached maturity.

Rare cytological aberrations. A number of chromosomal configurations were observed which were relatively uncommon in comparison with the bridges and acentric fragments which were used for comparing the effects of the treatments. Although the same or similar aberrations have been reported in the radiobiological literature, it seemed desirable to record their occurrence in barley as a contribution to studies on chromosomal mechanics.

In somatic mitosis: During the root-tip analyses for the frequencies of chromatinic bridges, care was taken to examine many of the prophase and metaphase nuclei for changes which would not necessarily be detectable at anaphase.

Few of the prophase nuclei had sufficient spreading of the chromosomes to permit cytological analysis. One exceptional cell is shown in figure 9. Two ring fragments and a number of other fragments are clearly in evidence. Because of the large number of fragments and the possibility that some of the chromosomes were dicentrics, it was not possible to determine whether or not the rings were centric or acentric. From studies on other stages, however, it is considered likely that they had centromeres.

Rings and dicentric chromosomes also occasionally were detected at metaphase. Photomicrographs of some of these rare cells are shown in figures 10 and 11. Dicentric chromosomes like those illustrated in figures 10 and 12 produce the chromatinic bridges recorded at anaphase.

Four of the 14 somatic chromosomes in barley have satellites; otherwise it is difficult to distinguish the chromosomes from one another. If by chance a translocation occurred between two satellited chromosomes, it would be possible to obtain a chromosome with satellites on both ends. The writers have had the good fortune to obtain a photomicrograph of such a chromosome (fig. 13).

Theoretically, if dicentric chromosomes are produced, tricentrics, quadricentrics, etc., should also occasionally occur. A tricentric and a chromosome with at least four centromeres are illustrated in figures 14 and 15.

In meiotic mitoses: A number of unusual chromosomal aberrations also were observed in the study of meiotic stages. Iso-chromosomes were the most commonly observed abnormality. In most cases two iso-chromosomes and six normal bivalents were present instead of seven bivalents (fig. 16). These two iso-chromosomes could have arisen in at least three ways: 1) The centromere of each of two homologous chromosomes may have been fractured, followed by the loss of one and a reduplication of the other arm from each homolog. The arms lost or reduplicated could not correspond; otherwise cells with seven bivalents should have been observed occasionally. Furthermore,

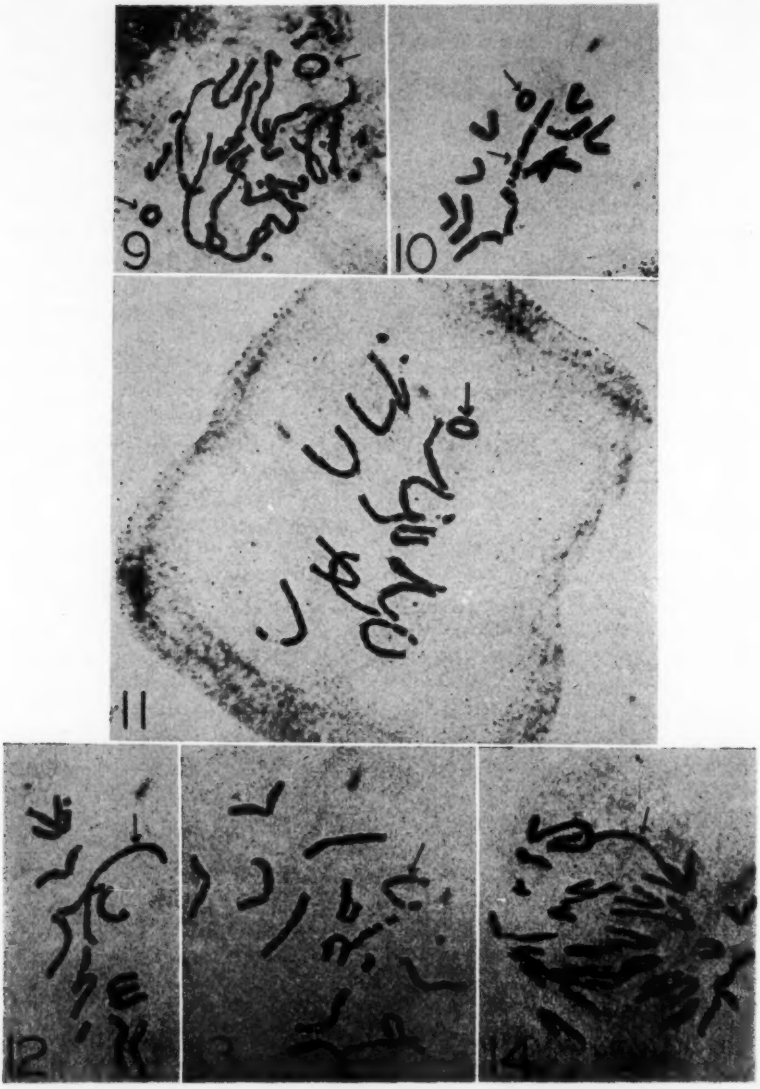

PLATE II

FIGURES 9 to 13.—Rare cytological aberrations observed at prophase and metaphase of somatic mitosis. 9, two ring chromosomes at prophase; 10, a ring chromosome and a dicentric at metaphase; 11, a ring chromosome at metaphase; 12, a dicentric at metaphase; 13, a translocation resulting in two satellites on the same chromosome.

FIGURE 14.—A tricentric at somatic anaphase.

if the same arms were lost, a deficiency would result which would probably be lethal to the affected cell in this diploid plant. 2) The iso-chromosomes could have resulted from interchanges between homologous chromosomes in such a manner that the new chromosomes had more or less identical arms (a possibility suggested by C. C. Mon). 3) The centromere of one chromosome could have been fractured and each half-chromosome reduplicated to form two iso-chromosomes. This would require the loss of the normal homolog to maintain 2n equal to 14.

Two iso-chromosomes were found in 16 unrelated X_1 spikes. Some 2,159 cells in these spikes were clearly analyzable, and not a single trivalent association was observed. Eighteen cells were observed in which pairing was not complete in both iso-chromosomes, and 39 cells in which pairing was not complete in one of the iso-chromosomes.

In one spike 708 cells were examined. Of these cells 706 contained two iso-chromosomes, but two cells each contained three iso-chromosomes (fig. 17). This indicates a possible instability of the iso-chromosomes during premeiotic mitoses.

In one instance two iso-chromosomes were found in a spike which also had an interchange (fig. 18). The translocation and the iso-chromosomes were apparently of independent origins, and were not associated in any of the pollen mother cells observed.

In two spikes a single iso-chromosome and an interchange were present. In one of the spikes a ring-of-four, five bivalents and one iso-chromosome were present, a total of 15 chromosomes (figs. 19, 20). A second anaphase in this plant is shown in figure 21. It is probable that one chromosome in the translocation is the homolog, say chromosome X, of the chromosome from which the iso-chromosome was produced. However, if such is the case it is difficult to explain the closed ring-of-four, unless it is assumed that it resulted from non-homologous pairing involving chromosome X. In the second spike a ring-of-four and an iso-chromosome were present, together with four bivalents and a univalent in some cells (fig. 22), or three bivalents and a ring-of-three in others (figs. 23, 24).

It is probable in the latter case that the univalent chromosome, which was occasionally associated with one of the bivalents to form a closed ring-of-three, was the homolog of the chromosome from which the iso-chromosome was produced, because no other configurations were observed. If this is the case it is apparent that we have a second instance, analogous to the one previously mentioned, of pairing between non-homologous chromosomes. Pairing between the iso-chromosome and the univalent was not observed, nor was a trivalent association between the univalent and one of the pairs ever observed.

It should be mentioned that non-homologous pairing at meiosis in barley never has been positively identified by the writers. The hypothesis of non-homologous association is tendered to account for the configurations mentioned above in preference to other explanations involving large deficiencies which, in barley, are almost certainly lethal.

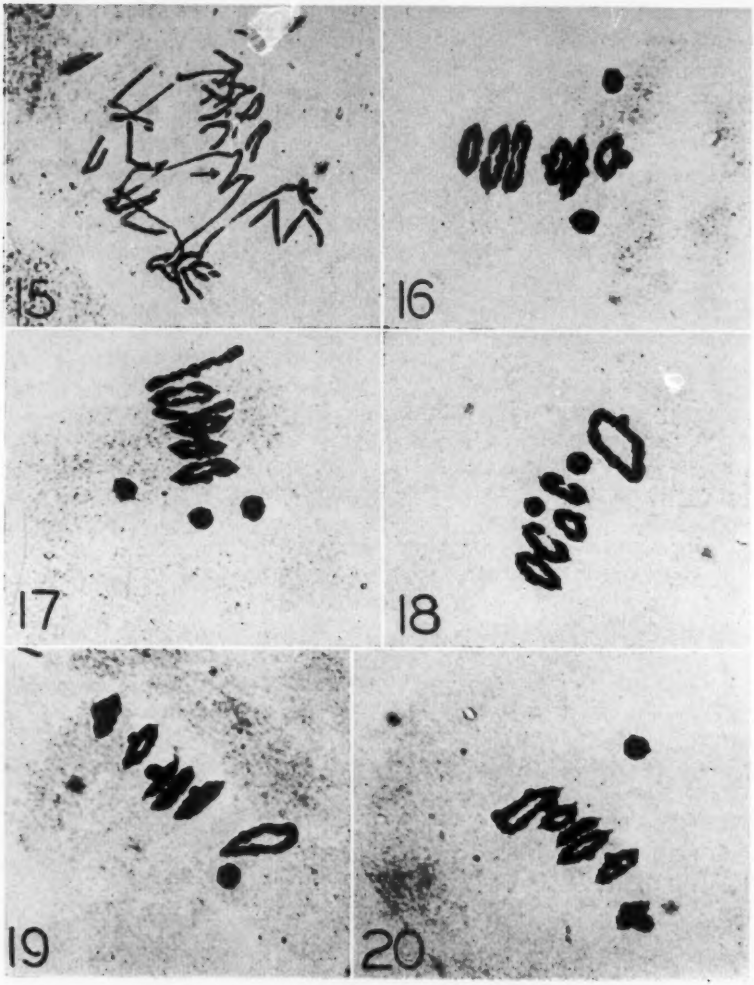

PLATE III

FIGURE 15.—A probable quadricentric at somatic anaphase. × 1000.

FIGURES 16 to 18.—Iso-chromosomes at first meiotic metaphase. 16, six bivalents and two iso-chromosomes. 17, six bivalents and three iso-chromosomes (from the same spike as 16); 18, four bivalents, two iso-chromosomes and a ring-of-four. × 1000.

FIGURES 19 and 20.—Ring-of-four, five bivalents and one iso-chromosome in pollen mother cells of a plant with 15 chromosomes. × 1000.

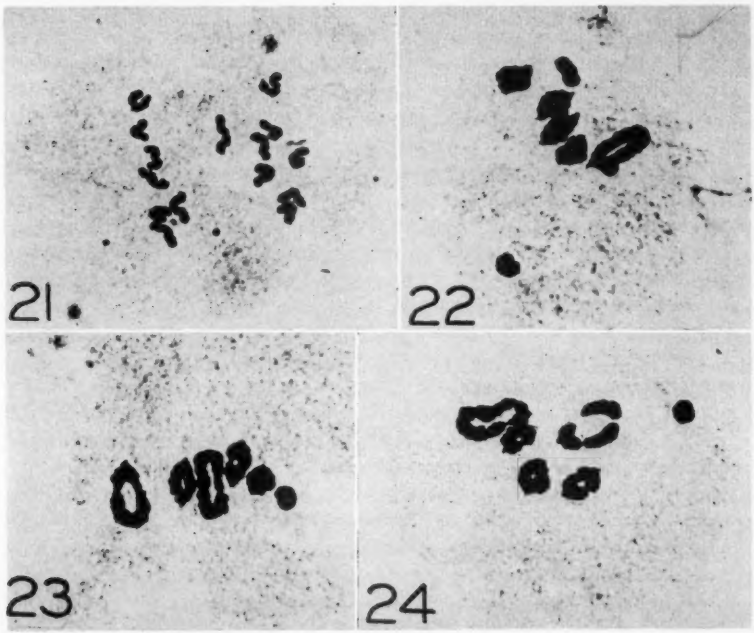

PLATE IV

FIGURE 21.—15 chromosomes at second anaphase in pollen mother cell of the same plant as shown in figure 20.

FIGURES 22 to 24.—First metaphase in pollen mother cells with an iso-chromosome, a ring-of-four, and a univalent which is associated with a bivalent to form a ring-of-three chromosomes in some cells. 22, ring-of-four, one iso-chromosome, one univalent, and four bivalents; 23, 24, ring-of-four, one iso-chromosome, ring-of-three and three bivalents. × 1000.

DISCUSSION

Most workers measure the biological effects of X-rays in terms of injury, chromosomal aberrations, or mutations. The term "mutation" is used herein in the narrower sense to refer to changes which are transmitted in frequencies comparable to naturally occurring mutations. A mutation then would not include aberrations which are detectable cytologically, but undoubtedly would include minute chromosomal changes, especially deficiencies. Not many organisms lend themselves to experimental procedures which permit measuring the effects of X-radiation by all these criteria. Perhaps that is the reason why it is generally assumed (or accepted as proven) that injury, chromosomal aberrations, and mutations are affected in a more or less parallel manner—if not causally related. SMITH and CALDECOTT (1948) obtained evidence to the contrary, and the present study was planned to obtain conclusive proof one way or the other on this point. Barley seemed to be a particularly suitable test organism because it can be used in so many ways for measuring the effects of irradiation. Furthermore, the influence of high temperature treatments applied to barley seeds either before or after X-radiation appeared to provide a means for determining whether or not injury, chromosomal aberrations, and mutations were necessarily closely associated effects of irradiation.

The results here presented prove beyond reasonable doubt that under the conditions of these experiments, X-ray-induced injury, chromosomal aberrations, and mutations are not correlated phenomena.

Injury symptoms were not closely correlated with the frequencies of either chromosomal aberrations or mutations. The lack of association between injury and chromosomal aberrations was particularly apparent in a number of tests in which a heat treatment following X-radiation resulted in increased injury, but decreased the frequency of bridges in root-tip cells and heterozygous interchanges in pollen mother cells by about 50 percent. However, in the summary of 11 tests, a slight decrease in injury, as measured by height of seedlings, resulted from this combination of treatments. The same lack of correlation was indicated by the fact that there was almost no difference in survival to maturity of seeds receiving X-rays alone, or the two combination treatments.

The lack of correlation between injury and mutation frequency was evident in the three tests where data on these criteria were obtained. This lack of association was most apparent when seeds were heated and then X-rayed. Under these conditions the injury (as measured by seedling height) to the seeds given the combination treatment was significantly decreased, and the mutation frequency significantly increased as compared with the seeds receiving X-rays only. Seeds treated with heat following the irradiation were sometimes injured more, and sometimes less, than the seeds subjected to X-radiation only. (Such variability was observed only when the treatments were applied in this sequence.) However, in all three years in which mutation data were obtained, the mutation frequency was higher in the material given the combination treatments than in the seeds receiving X-rays only.

Furthermore, there was no essential difference between the survival to maturity of seeds given either of the combined treatments and the seeds given X-rays only. That is, there was no correlation between survival to maturity and the mutation frequency.

Similarly, chromosomal aberrations and mutations were not closely correlated phenomena under the conditions of these experiments. The frequency of chromatinic bridges in root-tip cells and the frequency of interchanges in pollen mother cells of X_1 plants were reduced by combined heat and X-ray treatments, whereas the mutation frequency was increased. This lack of association was strikingly apparent whether or not the heat treatment preceded or followed X-radiation.

Experiments on Paramecium have demonstrated that even a small dose of X-rays which has neither visible nor division-retarding effects, affects the protoplasm (GIESE and HEATH 1948). The recovery from these effects is slow but is enhanced when the organism is supplied with food. This led GIESE to suggest that injury is overcome by replacement of X-ray-affected molecules. It also seems possible that such injury may be caused by the production of toxic substances or the partial destruction of enzyme or hormone systems which are essential to thrifty development. Whether or not any or all of these phenomena are responsible for the injurious effects of X-rays is unknown. But the fact that organisms can recover from X-ray-induced injury makes it seem likely that the injury is caused by some such temporary chemical disruption of the system. The manner in which heat treatments given before and after X-radiation may deter or enhance such postulated phenomena is a question.

A number of workers have speculated as to how temperature could affect frequencies of irradiation-induced chromosomal aberrations. There seems to be no exception to the rule that the frequencies are reduced by high, as compared with room or low, temperatures. This result appears to be independent of the test organism—having been found in Drosophila, Tradescantia, and barley. SAX and ENZMANN (1939) postulated that high temperatures facilitated the movement of broken ends of chromosomes and resulted in a reunion of broken ends in their original positions. They suggested that a heat treatment during or immediately after X-radiation would be effective in reducing the frequencies of detectable aberrations. The results of SMITH and CALDECOTT (1948) and those reported herein can be said to fit this hypothesis, because heat treatments either before or after X-radiation reduced the frequency of apparent breaks in the chromosomes. Also since oxygen tension influences the frequency of chromosomal aberrations resulting from ionizing radiations, it may well be, as pointed out by GILES (1950), that the effect of temperature on the frequency of X-ray-induced aberrations is an indirect one through an influence on the availability of oxygen.

Reports on the influence of temperature on the mutation frequency induced by X-radiation are not in agreement. In Drosophila the frequency is higher per "r" unit if the irradiation is applied at around 0°C than if applied at

about room temperature (MEDVEDEV 1935; KING 1947; NOVITSKI 1949; BAKER and SGOURAKIS 1950). This result parallels the effect of temperature on the frequencies of chromosomal aberrations (see above). The results reported herein, and those of SMITH and CALDECOTT (1948), contrast with these in that a high temperature either before or after X-radiation increased rather than decreased the mutation frequency. STADLER (1931) also reported that the mutation frequency in barley was lower per "r" unit if the irradiation was applied at about −68°C than if applied at room or higher temperatures.

If it is true that high temperatures promote restitution of broken chromosomes, the increase in the frequency of mutations from the combined heat and X-ray treatments reported herein might be accounted for by assuming that mutations arose at some points of breakage and restitution.

In the present study no attempt was made to determine if there is any reciprocal influence of the X-radiation on the effects of high temperature. If this influence is real, as indicated by the results of GIESE and HEATH (1948), CALDECOTT and SMITH (1948), and NYBOM (1950), an interesting avenue to the study of heat effects would be opened up. CALDECOTT and SMITH considered some of the possible mechanisms involved in such an influence.

The effect of infra-red on the frequency of chromosomal aberrations induced by X-radiation is apparently different from that of heat (SWANSON and HOLLAENDER 1946; SWANSON 1947, 1949). Since infra-red applied before or after X-radiation increases the frequencies of aberrations, the mechanisms involved in the effects of infra-red and heat must be different.

The frequency of detectable X-ray-induced mutations in *Aspergillus terreus* and *Trichophyton mentagrophytes* was increased by subjecting spores to infra-red before X-raying them (HOLLAENDER and SWANSON 1947; and SWANSON, HOLLAENDER and KAUFMANN 1948). It was suggested that this increase in mutations might be associated with an increase in chromosomal aberrations. However, in the present study there was a decrease in chromosomal aberrations resulting from the same treatments which increased the mutation frequency.

It is interesting to note that while the mutation frequency of X-rayed dormant seeds of barley was increased by pre- and post-treatment with heat, the relative proportions of mutant types produced was unaltered (table 6). That is, there was a proportional increase in all types rather than an increase in any one or a few classes of mutants. The data suggest that the effect of heat on the mutation process is merely to supplement a reaction initiated by X-radiation.

SUMMARY

Experiments were performed on dormant seeds of barley to determine whether or not injury, chromosomal aberrations, and mutations are necessarily correlated effects of X-radiation. The following treatments were

applied: (1) Control, (2) X-radiation (15,000r) alone, (3) A barely sub-lethal heat treatment—30 minutes at temperatures varying from 65°C to 85°C in different years, (4) X-radiation followed immediately by heat treatment, (5) Heat treatment followed immediately by X-radiation.

Effects of the treatments were measured by: (1) Injury—height of seedlings after 14 days growth, and survival to maturity, (2) Chromosomal aberrations—bridges in root-tip cells, and interchanges in microspores of X_1 plants, (3) Seedling mutants in X_2 progenies.

The following observations were made during the course of this investigation:

(1) Barely sub-lethal heat treatments had little if any effect on injury, chromosomal aberrations, or mutations.

(2) Such heat treatments applied immediately before or after the X-radiation had no apparent effect on injury as measured by survival.

(3) These heat treatments did influence injury in X-rayed seeds as measured by seedling height at 14 days. A heat treatment preceding the X-radiation significantly reduced the injury. If the heat and X-ray treatments were given in the reverse order, the results were variable; in some tests the injury was more, in other cases less, than resulted from X-radiation alone.

(4) Heat treatments applied either before or after X-radiation reduced the frequency of chromatinic bridges in root-tip cells by approximately half.

(5) The heat treatments reduced the frequency of interchanges in the pollen mother cells of X_1 plants by 30–40 percent.

(6) The heat treatments increased the mutation frequency in X_2 progenies by 30–50 percent. A heat treatment before increased the frequency more than a similar treatment applied after X-radiation.

(7) The types of mutants induced by the irradiation and the relative proportions of these types were apparently not altered by the pre- or post-treatment with heat.

(8) It was concluded that injury, chromosomal aberrations, and mutations are not necessarily correlated effects of X-radiation.

(9) Since high temperatures either before or after X-radiation influence the results of the irradiation in the ways observed, interesting possibilities as to the interrelationships of injury, chromosomal aberrations, and mutations are opened up. Some of these are briefly discussed.

(10) A number of photomicrographs of X-ray-induced chromosomal aberrations are presented and their cytogenetic implications discussed.

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A GENETIC INVESTIGATION OF ALLOMETRIC GROWTH IN HEREFORD CATTLE[1]

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BIOLOGISTS have long directed attention to problems relating to the determination of quantitative measures of body conformation and the genetic factors responsible for variation in conformation. Such variation in conformation between individual animals within a species is due to differences in the relative proportions of the various parts to each other and to the animal as a whole. D'ARCY THOMPSON (1917) pointed out that all but the simplest organisms reach their adult form by differential growth in different directions. Variation in conformation is the result of differences in relative growth rates of the various dimensions. A study of relative growth, or allometry, offers an approach to the problems of conformation.

Ramifications arising from the simple expression relating the size of a part to that of another part or the whole have been studied by investigators in the fields of theoretical and applied biology. In the present discussion reference will be made to a number of these investigations, most of which have been critically reviewed by REEVE and HUXLEY (1945), MEDAWAR (1945), and RICHARDS and KAVANAGH (1945).

The basic allometric relationship is that expressed by the equation

$$y = bx^a$$

in which y represents a part, x another part or the whole, and b and a are constants. The equation is either a parabola or hyperbola, depending on whether a is positive or negative.

In logarithmic form the equation becomes

$$\log y = a \log x + \log b, \text{ or}$$
$$Y = K + aX, \text{ where}$$
$$Y = \log y$$
$$X = \log x$$
$$K = \log b$$

Thus in logarithmic form the allometric equation is that of a straight line with slope a and Y intercept K.

Although the allometric equation had been used previously to express brain weight/body weight relations in mammals (SNELL 1891 and DUBOIS 1898, 1914) and birds (LAPICQUE 1898) as well as heart-body-weight rela-

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tions in birds (KLATT 1919), HUXLEY (1924) first used the formula to describe the relation of the growth of a part or organ to that of the whole organism and suggested that this equation might express a general law of differential growth. HUXLEY (1932) and others found a wide range of growth and size phenomena could best be described by the allometric equation.

The diverse evolution of the allometry concept has resulted in a confusion of terminology and symbolism. Recent suggestions for a standard terminology do not seem to be entirely adequate and have not been universally accepted. REEVE and HUXLEY (1945) and RICHARDS and KAVANAGH (1945) have summarized the terminology and symbolism in current use.

KAVANAGH and RICHARDS (1942) have shown that the data from most allometric studies may be classified into one of five types. These are:

Type A. Measurements of two variables X and Y are taken from time to time on the same individual and constitute the entire set.

Type B. A group of individuals as homogeneous as possible with respect to causative factors is chosen, and one set of measurements is taken on each individual. Such data actually portray relative size rather than relative growth.

Type C. A series of type B measurements is taken on the same group at successive intervals. In the usual treatment the arithmetic means $\overline{X}$ and $\overline{Y}$ are taken as the best representatives of the group values at each stage.

Type D. Measurements are made on a group of organisms without regard to differences between the developmental stages of the individuals, and a two-dimensional distribution is made of all the data for study.

Type E. This consists of a set of Type A curves for a group of individuals.

At present, it appears more satisfactory to give a clear statement about the class of data involved rather than a further elaboration of terminology.

KAVANAGH and RICHARDS (1942) considered several mathematical aspects of the allometric equation. They concluded: (1) the equation is dimensionally valid, a being the ratio of two specific growth rates (dy/ydt : dx/xdt) and, therefore, a pure number. When x is measured in U^i and y in V^j, the dimensions of b are $U^{-ia}V^j$. Since the value of b depends on the unit of measure, no unique biological significance or interpretation may be attached to it. (2) The relation between the parameters, $b = Be^{-ra}$, observed by HERSH (1931, 1934) is due to the mathematical treatment. (3) Contrary to LUMER's (1934) conclusions, whatever may be the curve of sigmoid growth followed by one of the variables, it is possible for the growth of the other to be sigmoid as well without invalidating the allometric formula.

HUXLEY (1924) first discovered the power equation, $y = bx^a$, empirically. Later (1932), in an attempt to establish a theoretical basis for the equation as a biological law, he derived the formula on the basis of assumptions about growth in general. A number of investigators (ROBB 1929; TWITTY 1930; TEISSIER 1934, 1937) have postulated different hypotheses to account for the allometric equation as a fundamental law of growth, but none of these has withstood critical analysis. It must be concluded that no satisfactory theoretical basis has yet been found for simple allometry. The allometric equation

must be considered as nothing more than a statistical expression and not an intrinsic biological law of growth. The parameters of the equation cannot be given a physiological interpretation. However, the almost universal applicability of the allometric equation to relative growth data cannot be overlooked. The equation may be considered as a measure of the resultant process or processes affecting relative growth, and as such may be looked upon as a law.

The present investigation arose from the desire to discover a quantitative measure of body conformation for beef cattle that could be used as a selection criterion in a breeding program designed to produce a desired type of beef carcass. Genetic improvement of body conformation of beef cattle has progressed slowly. This is partly due to difficulties of measurement and evaluation of beef-producing qualities. An even more important cause of slowness of progress may be the fact that investigators of beef form have considered conformation as a static characteristic. Thus the heritabilities of a number of body measurements relating to beef conformation have been estimated. Most of these have been tabulated by PHILLIPS (1949).

Since body conformation at any stage of development is the result of differential growth rates of the various dimensions, genetic studies of form should take account of the dynamic processes of growth. Attention should be centered on the processes leading to the assumption of the specific form rather than on the end products themselves. LERNER (1939) discussed the analogous problem in poultry and pointed out that: "Genetic studies of shape or conformation which do not take account of developmental rates address themselves to the method of inheritance of something that is the result of the interaction of a number of processes rather than to the inherited factors themselves. By means of allometric constants describing growth in different dimensions, we should be able to isolate the inherited factors themselves."

HUXLEY (1932) showed that the parameters of the allometric equation provide a simple numerical basis for comparison of growth patterns of parts, organs, and dimensions. When fitted to continuous growth data the allometric equation provides a quantitative description of the relation between the parts at any stage of development over the range fitted. A number of such equations describing the relation between pertinent dimensions and body size would yield a quantitative measure of conformation. Should variation in the value of the parameters be due to hereditary differences, there could be devised a selection index, based on the parameters for quantitatively changing body conformation.

The objectives of this investigation were:

(1) To determine whether the available relative growth data on Hereford cattle could be expressed by allometric equations;

(2) To estimate the parameters, provided the data could be expressed by allometric equations;

(3) To evaluate the allometric equation as a quantitative measure of body conformation; and

(4) To investigate the inheritance of the parameters of the allometric equation, thereby determining whether specified changes in conformation could be effected by using the parameters as criteria of selection.

MATERIALS AND METHODS

The animals used in this study consisted of 55 males and 52 females born in the University of California purebred Hereford herd at Davis. The data were obtained from records taken routinely as a part of the beef cattle breeding project. A series of nine measurements are taken on all animals in the herd at birth and every four months thereafter until two years of age. Subsequent measurements are made yearly until the animal leaves the herd. In actual practice the herd is weighed and measured once a month, so that there is some variation as to the exact age of each animal for each period measured.

The measurements include weight, height at withers, height at hook bones (tuber coxae), heart girth (*i.e.*, chest girth), round (distance from patella to patella parallel to the ground), length of body (horizontal distance from the point of the shoulder to the intersection with a line falling vertically from the posterior point of the tuber ischii), length of head, width of head, and width of hook bones (distance between the tuber coxae). Weight is taken on a normal fill to the nearest 2½ pounds. Heart girth, length of body, and round are measured with a corded linen tape. The remaining measurements are taken with calipers especially designed for the purpose.

It is evident that each of the dimensions measured is the result of a combination of measurements of organs and parts. HALDANE (see HUXLEY 1932, p. 81) pointed out that if the parts of an organ (*e.g.*, segments of a limb) show unequal constant differential growth ratios against a standard, then the whole organ cannot obey exactly the allometric law against the same standard and vice versa, since the sum of a number of expressions $b_r X^{a_r}$, with different values of a_r, cannot be identical with a single expression bx^a. TEISSIER (1934) argued that since the discrepancy is slight when the values of a are not very different, the theoretical difficulty is disposed of in practice. However, it is certainly important when the biological meaning is to be interpreted.

In the present studies it was not possible to determine whether the components of each measurement show equal constant differential growth ratios against heart girth. It would, however, be an unusual coincidence if they did. Hence, it is necessary to recognize this source of error in the values of the parameters.

PRELIMINARY ANALYSIS

The requirements of the present investigation were best fulfilled by a Type E (KAVANAGH and RICHARDS 1942) analysis of the data, as this permits the study of the growth curves of individuals. Heart girth, a good criterion of body size and subject to small error of measurement, was selected as a standard with which to compare the remaining measurements. Although it may have been desirable to use a longer period, limitation of the data re-

quired the use of the range from birth through sixteen months of age. This shorter range does not affect the results seriously, as will be shown later.

In order to estimate graphically whether the data could be represented by the allometric equation a random sample of the animals was drawn, and for each a double logarithmic plot of the eight available measurements against heart girth was made. A typical set of double logarithmic plots is shown in figure 1. All the points except the first or birth measurements fell along a straight line, within the errors of measurement. It was concluded, therefore, that with the restriction discussed below the allometric equation provides a satisfactory quantitative description of beef conformation.

The first point was far out of line, indicating a disturbance in the value of a between birth and four months of age. PONTECORVO (1938, 1939) fitted the allometric equation to a foreleg length/height at withers relation in cattle and found some evidence that the most rapid growth of the trunk, relative to the foreleg, occurs just after birth. He pointed out that the young of most grazing mammals are born with relatively long limbs. Therefore, during embryonic development a must be greater than unity, and about the time of birth it assumes a value less than unity.

Measurements were taken at monthly intervals on a number of calves until they were four months of age, and a double logarithmic plot of the data was made. It was evident from these graphs that a changes markedly between birth and one month of age, but approaches a stable value after that time. The available data do not provide information as to the exact nature of this change. Further data, discussed later, indicate that a continues to change gradually throughout growth. These data suggest that a approaches at least two widely separated values during the life of an individual. Fetal values of a measure the relative growth processes that best adapt the individual to embryonic development, parturition, and early post-partum existence. Associated with the change from intra-uterine to external environment is a change in the value of a. The change is initiated during a short interval occurring just before and immediately after birth. By the time the animal reaches one month of age, a has approached a stable post-natal value.

STATISTICAL ANALYSIS

In fitting the data the first or birth measurements were omitted, since their wide divergence from the other values would influence the estimates of the parameters markedly and detract considerably from their accuracy in describing conformation. Therefore, all the parameters were estimated from four pairs of measurements corresponding to 4, 8, 12, and 16 months of age.

KAVANAGH and RICHARDS (1942) have shown that for the individual curves of a Type E analysis, the most probable values of b and a are those obtained by use of a least squares technique that accounts for error in both variables.

Theoretical justification for the use of the least squares technique arises from the condition that the errors be normally distributed and independent

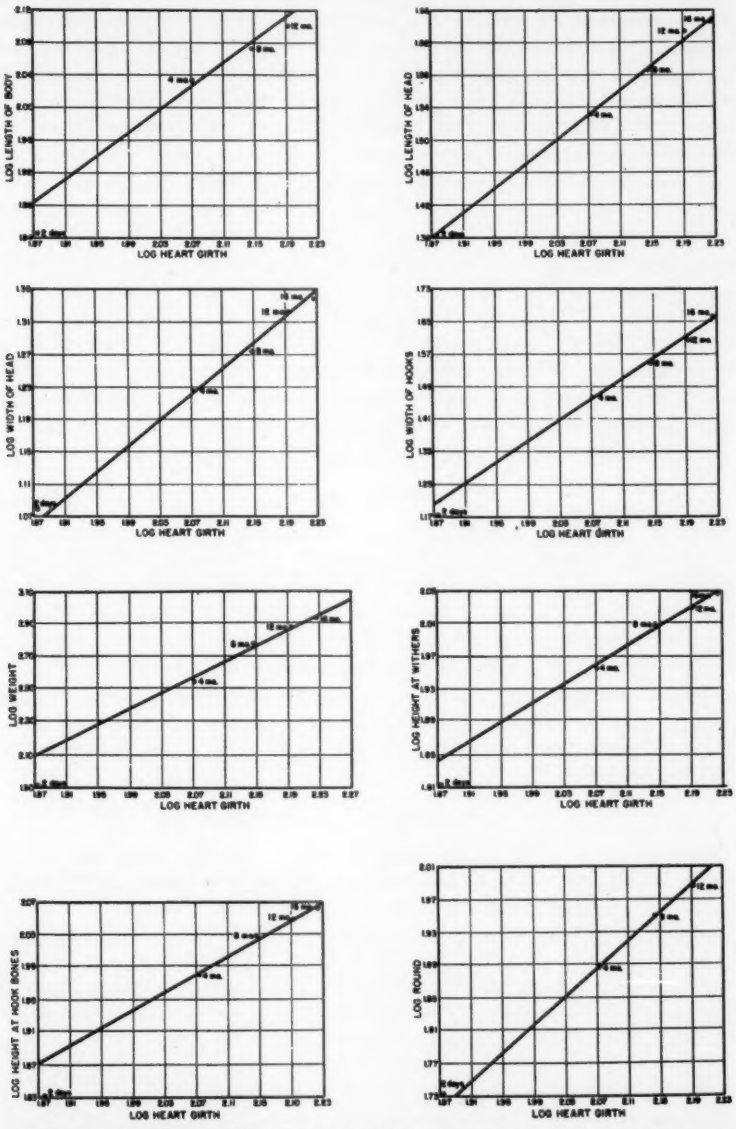

FIGURE 1.—Double logarithmic plots of eight body measurements against heart girth of a typical female.

of each other. GREGORY (1933) published the results of 100 successive measurements of the round of an individual cow. These data were tested for normality by the method of SNEDECOR (1946). The analysis indicated that the measures were normally distributed. It appears reasonable to assume that the other measurements also follow a normal distribution. Independence of the errors was assumed on the basis of the objective method of taking the measurements.

If the logarithmic form is fitted it is necessary to consider the question of weighting, since the deviations from the parabola are not equal to the deviations from the straight line logarithmic form. However, KENNY (1947) stated that the discrepancy usually does not affect the fit seriously. KAVANAGH and RICHARDS (1942) drew evidence from the data of NEEDHAM (1934) and TEISSIER (1934) and showed that there would be little difference between the correctly weighted and the unweighted methods of treatment of the logarithmic form. For the purpose of this study, the increased precision would not justify the additional labor of properly weighting the logarithmic form. The logarithms of the measures were fitted by the method of least squares to account for error in both variables suggested by KENNY (1947).

VARIATION IN a

In those instances where the data were available, parameters were estimated on the basis of six pairs of observations, *i.e.*, through 24 months of age. Comparison with those calculated on the basis of measurements through 16 months indicated that they were estimates of different parameters. The difference was not large enough to be of practical importance to the results of this investigation. It appears that the values of a and K are not actually constant, but are gradually changing throughout development. These analyses are in general agreement with those of LERNER (1938), who found variations in the differential growth rate of the leg length against body weight in the domestic fowl.

VALUES OF a AND K

Mean values of a and K are shown in table 1. The data were analyzed by means of analysis of variance to determine (1) sex differences and (2) intraclass correlations to be used in estimating heritabilities.

Significant differences in mean values of a and K between the sexes were found for height of hooks, round, width of head, and width of hooks relationships. (Height at hooks/heart girth, etc., are referred to simply as height of hooks, etc.)

Height at hooks

The data indicate that the Y intercept, K, is greater for males, but the value of a is larger for females. Thus, height at hooks has a higher relative growth rate for females than for males. At birth females are slightly higher at hooks than males of equal size of heart girth; throughout development they maintain a higher relative growth rate increasing the divergence. Simulta-

neous solution of the two equations shows that the lines intersect when heart girth has a value of 13.4 centimeters. That would occur during embryonic development. Since it has already been established that a changes markedly during the period from just before birth to one month of age, extrapolation of the data into the range prior to one month of age is not valid for purposes of biological interpretation. Thus it is clearly established that in these data a and K are not primary biological factors but possibly are measures of one. As such, they may prove useful criteria of selection.

TABLE 1

Mean values of a and K.

		Grand mean	Mean males	Mean females	Difference
Weight	a	2.660	2.662	2.657	.005
	K	−2.980	−2.976	−2.984	.008
Height at hooks	a	.571	.553	.590	.037*
	K	.789	.829.	.747	.082*
Round	a	.747	.787	.706	.081**
	K	.351	.274	.432	.158**
Head length	a	.737	.749	.725	.024
	K	.004	− .020	.030	.050
Height at withers	a	.662	.614	.631	.017
	K	.653	.673	.631	.042
Length	a	.861	.854	.868	.014
	K	.220	.242	.197	.045
Head width	a	.704	.742	.663	.079**
	K	− .228	− .304	− .148	.156**
Width hooks†	a	1.162	1.110	1.223	.113**
	K	− .956	− .851	−1.078	.227**

* 5 percent level of significance.
** 1 percent level of significance.
† Means based on 48 males and 41 females.

Round

The value of the Y intercept is larger for females, but males have a greater value of a. The two curves intersect when the heart girth measures 86.4 centimeters. This corresponds to a little more than one month of age. It appears that at birth females have a greater round relative to heart girth than males, but that the males have a higher relative growth rate, so that at about one month the two sexes have the same relative size of round; thereafter the males develop a larger round, relative to heart girth, than the females.

Unfortunately, comparable data for steers are not available. However, should their growth pattern be more similar to the males than the females, it would explain in part the general preference of buyers for fat steers rather than fat heifers.

Width of head

The Y intercept is larger for females, but a is greater for males. The two curves intersect when heart girth has a value of 93.2 centimeters, which corresponds to 6 to 8 weeks of age. Thereafter, males are relatively wider of head than females, the divergence increasing until mature proportions are attained.

There is no significant difference between the two sexes as regards relative growth of head length. Males and females thus attain their characteristic different mature proportions by different relative growth rates in head width only. Thus in a group of mature animals of the same heart girth males and females would have heads of equal length, but males would have much wider heads than females.

The mean value of a for head length is 0.737 for both sexes. For head width the value for males is 0.742 and for females 0.663. As maturity progresses the ratio of head length to head width becomes progressively larger for females and smaller for males. Thus the conformation of an animal's head, *i.e.*, the ratio of length/width is in a sense a measure of maturity. This may explain why practical breeders give the shape of the head so much consideration when selecting sires or feeders. Table 2 shows how the ratio of head length over head width changes with advancing maturity in both sexes.

TABLE 2

Ratio of head length to head width for different values of heart girth.

Heart girth	Males	Females
cm.		
65	1.988	1.933
110	1.982	2.009
140	1.980	2.045
165	1.979	2.070
180	1.978	2.083
190	1.977	2.093
200	1.976	2.100

Width of hooks

Males have the larger intercept but females the greater growth ratio. Intersection of the curves is at a value of heart girth equal to 99.9 cm., which corresponds to an age of 3 to 4 months. Thus, at birth males are slightly wider at hooks, but the relative growth rate of the females is higher. At about 3 to 4 months the females become wider at the hooks than males of the same heart girth measurement.

EFFECT OF INBREEDING ON THE VALUE OF a AND K

In order to estimate the effect of inbreeding on the value of a and K the regression of parameter value on coefficient of inbreeding (WRIGHT 1922) was calculated in each case. The coefficients of inbreeding ranged in value from 0.00 to 0.328, with an average value of 0.073.

None of the regression coefficients were significantly different from zero at the 5 percent level. Insofar as the limited data can be interpreted, there is no evidence of any effect of inbreeding on allometric growth in the population.

INTRA-CLASS CORRELATION ANALYSIS OF a AND K

Intra-class-half sib correlations for a and K were determined by analysis of variance and are shown in table 3. Sex differences were found for height at hooks, round, head width, and width of hooks relationships. The sex difference between values of a for round was just significant at the 5 percent level, the remaining differences being non-significant. A biological explanation of this difference is not immediately apparent. Since, with the exception mentioned, the differences noted between sexes were not significant the data for the two sexes were combined by the method described by WHATLEY (1942).

TABLE 3

Values of half-sib intra-class correlations.

	Males		Females		Both sexes	
	a	K	a	K	a	K
Body weight					.10	.10
Height at withers					.16*	.16*
Height at hooks	.28**	.28**	.25*	.26*	.25[2],**	.25[2],**
Length					.16*	.15*
Round	.18*	.17	−.06	−.04	.08[2]	.07[2]
Length of head					.31**	.32**
Width of head	−.13	−.13	.06	.07	−.02[2]	−.02[2]
Width of hooks[1]	.15	.17	.15	.15	.13[2],*	.13[2],*

[1] Means based on 41 females and 48 males.

[2] Pooled values for sexes based on method of combination described by WHATLEY (1942).

* Significant at 5 percent level.

** Significant at 1 percent level.

The value of the correlation for a and K for round measurement and head width is not significantly different from zero, and that for weight is just short of the 5 percent level of significance. The remaining correlations are all significantly different from zero—those for height at hooks at the 1 percent level and those for height at withers, body length, length of head, and width of hooks at the 5 percent level.

ESTIMATES OF HERITABILITY OF a AND K

The average genetic relationship among all half sibs in the population is 0.326. Heritabilities were estimated by multiplying the values of the intra-class correlation by the reciprocal of the average genetic relationship. These estimates are given in table 4. Significance of these estimates is determined by the significance of the intra-class correlation. WHATLEY (1942) pointed

out that there is very little dominance or epistatic correlation in heritability estimates obtained in this way. However, the small number of sires involved gives rise to a large sampling error.

Errors in estimates of heritability in either direction cannot be recognized with certainty unless they are less than zero or greater than unity. The estimates for head width and head length are apparently both in error. The heritabilities of a and K for head width are probably zero, while those for head length are quite high. It is puzzling that the variability of the relative growth rate of width of head is not at all influenced by additive gene action, but that variability of length of head is due almost entirely to additive gene action.

The generally high value of the heritability estimates is at variance with the results of investigations with the domestic fowl. LERNER (1943) summarized the results of four generations of progeny test selection involving 320 offspring for high and low values of a in a tarsometatarsus/body weight comparison and concluded that the genetic variability of the relative growth pattern in the stock used was either non-existent or so low as to preclude its demonstration by the method of selection in opposite directions employed.

TABLE 4

Heritability estimates.

	Weight	Height at withers	Height at hooks	Length	Round	Head width	Head length	Width hooks
a	.30	.50*	.75**	.48*	.23	−.08	.96**	.40
K	.32	.50*	.77**	.45*	.23	−.06	.97**	.40

* Significant at 5 percent level.
** Significant at 1 percent level.

LERNER's comparison was made with weight as a standard of body size while heart girth was used in the present investigation. The estimates of the half-sib correlation of a and K for the weight/heart girth relation were just short of the 5 percent level of significance, and thus it is questionable whether the actual heritability differs from zero. This suggests that relatively large measurement errors and random fluctuation in weights (as contrasted with linear measurements) might increase the environmental portion of variance of a and K to the extent that heritability of a and K becomes so low as to preclude its demonstration. It was, in fact, consideration of this possibility that led to the use of heart girth as a standard of body size in this investigation.

The hypothesis that heritability of a and K would be low or zero when body weight was taken as the standard was tested. The heritabilities of a and K for height at hooks are 0.76 and 0.77 respectively. Heritabilities for a and K for a height of hooks/weight relation were computed. In this instance a least squares technique accounting for error in one variable only was used to estimate a and K. This method used by LERNER increases the environmental portion of the variance of the parameters. The heritabilities of a and

K thus computed were not significantly different from zero. It would therefore seem that weight is not a satisfactory measure of body size when used as a standard for allometric growth studies, which may account for LERNER's failure to obtain gains under selection.

RELATION BETWEEN a AND K

The question of possible mathematical relationships between the parameters has been considered. HERSH (1931, 1934) observed the relation $b = Be^{-rs}$ in the values obtained from his study of facet number in bar-eyed Drosophila and in a study of relative size in various species and genera of titanotheres. In this equation B and r are constants and e is the base of the natural logarithms. LUMER (1936) pointed out that this relation is the necessary and sufficient condition that the relative growth curves shall all pass through a common point. KAVANAGH and RICHARDS (1942) studied double logarithmic plots of HERSH's data and pointed out that because of the unit of measure and because the organs measured were all of roughly comparable size, the points were grouped in a fairly small area of the plane compared with the distance to the line $X = 0$. Since the numerical value of K is the value of Y when $X = 0$ determination of K from the data represents extrapolation a considerable distance beyond the data and an approximation to LUMER's condition that all lines pass through a common point. The distance to the line $X = 0$ depends on the unit of measure of x. The line $X = 0$ can be moved closer to or even into the group of points by taking a larger unit of x and as the degree of extrapolation diminishes or disappears the relation between the parameters vanishes. KAVANAGH and RICHARDS (1942) present a rigorous mathematical demonstration of these arguments.

The relatively small unit of measure of x (heart girth in centimeters) employed in this study results in an extreme degree of extrapolation, and a close approximation to LUMER's condition that all lines pass through a common point. As a consequence, K is largely an inverse measure of a and most of the variation in K is merely a magnified inverse of the variation in a. This is illustrated by the close similarity between values of the half-sib correlation for a and K (table 3). Phenotypic, genetic, and environmental correlations between a and K were estimated. The phenotypic correlations were calculated directly from the raw data, and the genetic and environmental correlations were evaluated by the method of covariance analysis described by HAZEL, BAKER and REINMILLER (1943). These correlations all approached or were actually estimated as –1.0 as would be expected. It can be concluded therefore, that a and K are measures of the same biological phenomenon when determined by a type E analysis employing a relatively small unit of measure of x. These observations differ from KAVANAGH and RICHARDS (1942) conclusion that, since the value of b depends on the unit of measure no unique biological significance or interpretation may be attached to it. Since the actual value of b or K depends upon the unit of measure it is necessary that the same units be used for different values of K to be comparable. With this restriction it would seem that both a and K are valid measures of the same underlying

biological phenomenon. Practical consequences of this condition with respect to selection will be discussed later.

DISCUSSION

The relative growth data analyzed in this study can be represented by allometric equations. There are, however, some limitations that should be considered in order to interpret the biological significance of the results.

WRIGHT (1918, 1932), NELSON and LUSH (1950) and others have concluded from studies of inheritance of body size that the greater part of variation in a measurement within a race or breed is due to general size factors, *i.e.*, factors which affect all parts of the body approximately proportionately, and only a small portion of it is due to special or group factors. Wide differences in relative proportions or conformation within the Hereford breed are obvious, and it follows that they must have resulted from differential growth in different directions. The generally high heritability estimates obtained in this study indicate that some of these variations are in part the result of additive gene action. It should be emphasized, however, that the allometric equation when fitted to relative growth data of the type used in the study, exclude virtually all differences in general size. Therefore, it is necessary to consider the problem of general size independently but simultaneously with that of relative size. This is particularly true as regards selection practices.

The equation was fitted to Class E data as defined by KAVANAGH and RICHARDS (1942). Theoretically the allometric equation can be considered as adequately describing the relationships only when the discrepancy between calculated and observed values is within the limits of measurement error. Inspection of a number of graphs indicates that for the range fitted (4 through 16 months) this requirement is fulfilled and that the allometric equation provides a satisfactory description of beef cattle conformation. There is evidence that this condition would not hold over a longer range. First, there is the marked change observed in the value of a between birth and one month of age. There is also the observed difference between values of a calculated on the basis of 4 months through 16 months and those calculated on the basis of 4 months through 24 months. This difference indicates a changing value of a, so that any extrapolation beyond the range fitted will not give an exact representation of the relationships. The discrepancy will not be so marked if one extrapolates into the range between 16 months and maturity, but will be more serious as one extrapolates beyond the lower limit at one month.

Although these limitations make a physiological interpretation difficult, they do not lessen the usefulness of the equation for describing conformation. The description is nearly exact over the range fitted. The equation describes the relation between the two dimensions at any given time during development and the changes that take place during development. It is possible, by suitable combinations of the equations, to describe three-dimensional relationships and changes through any desired planes. It is also possible to compare any two measurements directly by eliminating the common standard, or to study changes in the relation between two of the measurements as the stand-

ard changes during maturity. Thus, the allometric equation, fitted to relative growth data, would appear to give a satisfactory quantitative description of conformation.

A major objective of this study was to determine the usefulness of the allometric equation as an aid in selection for changes in conformation. Since it has been demonstrated that the allometric equation is adequate for a quantitative description of conformation, the problem is reduced to determining whether the parameters can be modified by selection. In order to gain information on this point estimates of the heritabilities of each of the parameters were made. The data were amenable to the half-sib correlation method used.

Heritabilities of a and K for three of the relations were not significantly different from zero. For two of these, weight and round measure, it would appear that environmental contributions to the total variability are so large as to reduce the heritability. Both weight and round are influenced markedly by the plane of nutrition, while the effect on bone growth is not so great. This would be a source of much of the environmental variability.

The head relationships are puzzling. Heritability of the parameters for head width are almost certainly zero, while heritability of head length is estimated at unity. It seems hardly reasonable that such an outcome could be due to sampling error. It is more likely that the results are due to some peculiarity of the development of the bones of the skull.

In 1934 BRASH (see REEVE and HUXLEY 1945, p. 135) concluded from his studies with madder-feeding that increase in size of the skull is due primarily to external surface accretion and internal surface absorption rather than to growth of the ends of individual bones.

It seems, therefore, that no simple concept of multiplicative or additive growth can be applied to explain changes in skull proportions; nevertheless, the data for head dimensions appear to fit the allometric equation as well as the data for the other dimensions. One obvious and important result of differences in heritability of relative growth rates of the two dimensions of the head is that any change in conformation of the head must be effected by a change in length only, even though the factors controlling sexual dimorphism of head shape operate on width alone.

The heritability of the parameters of the remaining comparisons indicates that considerable change in conformation could be effected by selection for high or low values of the parameters. The data indicate that the parameters of the allometric equation would be useful criteria of selection for changes in body conformation in those instances where comparisons are between measures of linear skeletal development. In instances where muscular and fatty tissues are involved, it would be necessary to maintain a uniform plane of nutrition and to keep the environmental factors as nearly constant as possible. If this could be accomplished satisfactorily, the parameters of the allometric equation expressing such relationships might be usable as criteria of selection.

As previously indicated, the fact that a and K are measures of the same biological phenomenon gives rise to certain practical consequences with respect to selection. The relationship between a and K cannot be modified by

selection, *i.e.*, selection for one parameter will elicit or automatic correlated response in the other. Thus, it will not be possible to increase or decrease the value of K without a correlated decrease or increase in a. Although it is not possible to change a–K relationships by selection, it is possible to modify the a–K complex by selection either for a or K. Thus selection for either parameter alone is as effective as selection for both. Ease and simplicity of determination and manipulation will dictate the choice in any particular instance.

These considerations suggest the possibility of using a simple ratio taken at a standard body size as a criterion of selection. A simplification of this type would aid materially in actual breeding programs. The generally high heritabilities for various body measurements reported in the literature (PHILLIPS 1949) would lend support to the possibility of using a simple ratio. However, a simple ratio, or single plot, does not define a unique estimate of either parameter, and therefore it is not possible to determine the pattern of relative growth. A single ratio, therefore, would not be a suitable criterion of selection.

At least two points must be measured to define a unique genotype for the a–K complex. The minimum number of observed points compatible with the required degree of accuracy must be determined in each instance. In this connection, it is also necessary to consider the optimum age at which to practice selection. From the standpoint of the practical breeder the problem is to determine the minimum number of observations and the earliest age range over which to take them. A detailed analysis of this question was not possible, but several pertinent points are worthy of mention.

The nature of the change in value of a throughout growth should be investigated. Unless the change is marked, the effects will be small. Possibly a more important factor in determining the minimum number of points required is the effect of a reduced number of observations on the heritability estimates of a and K.

The degree of maternal effect involved in the inheritance of the parameters also has a bearing on the optimum time at which selection should be practiced. The half-sib correlation method used to evaluate heritability in this study does not include maternal effects, and sufficient data are not available at present to estimate them. However, the possibility of the existence of maternal effects cannot be overlooked. If present, maternal effects would reduce the rate of gain expected from mass selection and progeny testing.

SUMMARY

An investigation was made to determine whether relative growth data obtained from the University of California purebred Hereford herd could be represented by the allometric equation. The data consisted of a series of nine body measurements taken at birth, 4, 8, 12, and 16 months on 55 male and 52 female progeny of ten sires. The measurements include weight, height at withers, height at hooks, heart girth, round, length, width of head, length of head, and width of hooks.

Heart girth was selected as a standard of body size, and each of the remaining dimensions compared with it in a series of Type E allometric equa-

tions. For all such comparisons the value of a changes markedly between birth and one month of age. After that time it remains nearly stable, changing only slightly.

It was found that the data could be represented by the allometric equation over the range fitted and that the parameters give a simple numerical description of the relative growth patterns of the dimensions studied. The allometric equation provides a satisfactory quantitative description of body conformation.

A sexual dimorphism in relative growth pattern was noted for height at hooks, round, head width, and width at hooks. It was found that with advancing maturity the ratio of head length to width becomes progressively smaller for males and larger for females. It is suggested that this ratio may be a criterion of rate of maturity.

Heritability of a and K was estimated by the half-sib correlation method. These estimates indicate that in many cases the parameters of the allometric equation can be changed by selection. In general, the parameters of equations relating linear skeletal dimensions are heritable to a higher degree than those involving muscular or fatty tissues.

The heritabilities of the parameters for width of head did not differ from zero, but those for length of head were very highly heritable. An explanation is not immediately apparent.

An inverse mathematical relationship exists between the parameters. The practical consequences of this relation are that it is not possible to change the relationship between a and K by selection, but that it is possible to change the a–K complex by selection for either parameter alone.

It may be concluded that the allometric equation offers a quantitative description of beef cattle conformation, and that conformation might be changed in a specific manner by selection for desired values of the parameters.

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THE ANALYSIS OF TETRAD DATA [1, 2]

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THE theory of four-strand crossing over was founded by JANSSENS (1909). He arrived at his theory in an attempt to explain tetrads in which all four cells are different. None such had been observed at that time but JANSSENS believed, by a happy metaphysical insight, that since germ cells were always produced in groups of four, the four must be all different. It is curious that when tetrads were isolated and four-type tetrads obtained, their significance was often missed. A useful summary of the types of tetrad produced by various crossovers has been given by WHITEHOUSE (1942, 1949).

Haploid characters among the bryophytes, green algae, and fungi have been used almost exclusively in tetrad analyses although flowering plants would seem to offer excellent material. In many flowering plants, including nearly all the Ericaceae, pollen is shed in tetrads. If individual tetrads were placed on styles there should develop, with or without hormone treatment, four seeds corresponding to the four pollen grains of the tetrad.

The purpose of the present paper is to show what information can be obtained from tetrad analysis and to analyze some recent published data.

Tetrads can be classified into ordered and unordered kinds. In ordered tetrads the pairs of cells resulting from the first meiotic division can be distinguished and hence the second division segregation frequency for any gene pair estimated. In organisms which form spores in a linear order such as many ascomycetes and the smuts, they are represented by the top two or four spores and the bottom two or four. In organisms in which the tetrads form a square and in which the second meiotic division spindles form a cross, they are represented by spore pairs along the diagonals, which is probably the case in chiastobasidial basidiomycetes. Another type of ordered tetrad is represented by *Saccharomycoides ludwigii*. Here the second meiotic division spindles are parallel and at about the same level so that one daughter cell from each goes to the top and one daughter cell from each goes to the bottom (WINGE 1947). The top and bottom pairs of cells are not sister cells but are, so to speak, cousin cells. In this case the second division segregation frequency for a locus is twice the frequency of tetrads in which the top pair and bottom pair of cells have similar alleles.

In unordered tetrads spore pairs cannot be distinguished by their spacial position. In some cases they can be distinguished by other means. In organ-

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isms having a sex chromosome sex provides a convenient marker where it is known that the sex locus is always pre-reduced, this method has been used in *Sphaerocarpus* (KNAPP 1937). A gene so close to the centromere that practically no crossing over takes place between it and the centromere would also provide such a marker. The extra information that can be obtained from ordered tetrads or from unordered tetrads with a marker concerns crossing over in intervals bounded by a centromere. Information about the position of the centromere can, however, be obtained from any kind of tetrad so long as at least three gene loci are available.

One of the advantages of tetrad analysis over the analysis of random spores is, then, that it gives information about the position of the centromere. Another advantage is that it provides more precise information about interference. With tetrads interference can be estimated with as few as two gene loci, and when more loci are available chromatid interference can be distinguished from chiasma interference. With random spores chromatid interference can only be detected by the exceptional occurrence of recombination values in excess of 50 percent. Finally, in multi-loci crosses the frequencies of crossovers in each interval and of simultaneous crossovers in two or more intervals can be directly estimated. Indirect estimates of these can also be made with random spores (LUDWIG 1938; WEINSTEIN 1936) if chromatid interference is assumed absent. For most purposes these estimates are fortunately not required but for some they are (CHARLES 1938).

When linkage is estimated from recombination frequencies the collection of spores in tetrads is less efficient than random collection. If R is the frequency of recombination per strand between two linked loci, the variance of the estimated linkage value V_r, is $[R_r(1-R_r)]/n_r$ where n_r is the total number of spores collected randomly. When the spores are collected in tetrads the variance of the estimated linkage value V_t is $(R_t - 2R_t^2 + N)/2n_t$ where n_t is the total number of tetrads and N is the frequency of tetrads having all four spores showing recombination. This formula is equivalent to that given by MATHER and BEALE (1942) and L. J. SAVAGE has pointed out to me that although these authors arrived at this formula by an approximate calculation, an exact calculation gives the same result. If data from random spores and from tetrads are to be pooled the results from each should be weighted by the reciprocal of their variances so that the pooled estimate of recombination is $[R_r(1/V_r) + R_t(1/V_t)]/[1/V_r + 1/V_t]$ or $(R_rV_t + R_tV_r)/(V_r + V_t)$. Two random spores give about as much information as all four spores of a tetrad since the variance of R_t is about twice that of R_r. An example of the use of such weighting is provided by data from *Neurospora* (SHENG 1951) where linkage between the loci ***nd*** and ***al*$_2$** was estimated from 172 random spores and 24 tetrads. The four spores of a tetrad were apparently given the same weight as one single random spore to arrive at the mean value 20.5. By the above method $V_r = 0.000961$, $V_t = 0.00385$ and the mean value is 19.6. This disadvantage of tetrads is mitigated by the fact that if three spores of a tetrad are known the fourth can always be deduced and if only two spores

are known but those two have similar alleles for all but one of the characters involved, then the other two can be deduced.

CLASSIFICATION OF TETRADS

In the following discussion the centromere will be considered as a locus comparable to a gene locus. From this point of view unordered and ordered tetrads can be analyzed by similar methods. Pairs of loci will be classified into two-type parental (P), two-type non-parental (N), and four-type (F) in which there are 0, 2, and 4, recombinants in the tetrad respectively. This classification reduces the number of classes and simplifies analysis. Where one of the loci of a pair is the centromere then P and N cannot be distinguished and F represents the incidence of second division segregation of the gene locus. Whereas with random spores, there is only one independently variable frequency for each pair of loci, parentals or non-parentals, with tetrads there are two independently variable frequencies. It is often useful to know the number of classes of tetrad that can be expected from a cross with a certain number of factors, say x. The total number of possible arrangements of the four spores resulting from meiosis is 6^x. The number of classes of ordered tetrads, pooling only those classes that give no information as to crossing over is $(5 \times 2^x + 6^x)/8$. The number of classes of unordered tetrads $(6^{x-1} + 3 \times 2^{x-1})/4$, and if all pairs of loci are classified as N, P, or F, then the total number of classes is a bit more difficult to calculate but for the first ten values of x the series is 1; 3; 11; 48; 236; 1,248; 6,896; 39,168; 226,496; 1,325,577 (appendix I). The classification into N's, P's, and F's reduces the number of classes to be distinguished considerably. The information lost is, at worst, a failure to distinguish between the two types of 3-strand double crossovers (PAPAZIAN 1951).

With this scheme the detection of crossovers and the strands involved is simplified. For any three loci the types of crossovers corresponding to various combinations of N's, P's and F's are given in table 1. In the first three columns if an N occurs instead of a P, a four-strand double crossover is indicated instead of no crossover. It is impossible to have an N for all three pairs of loci so that by substituting N's for P's only three additional classes can be added to the first column and one additional class to the second and third column.

TABLE 1

Classes of tetrad produced by various kinds of crossing over.

Crossovers	None	Single in I	Single in II	Double, in I and II		
				2-str.	3-str.	4-str.
Loci pairs: A & B	P	F	P	F	F	F
B & C	P	P	F	F	F	F
A & C	P	F	F	P	F	N

The three loci lie in the order A–B–C with two intervals I and II respectively. P = 2-type parental, N = 2-type non-parental, F = four-type.

The table then accounts for the 11 possible classes with three factors. Where one locus is the centromere, for example the *A* locus, and the two gene loci lie in the same arm of the chromosome, two- and four-strand double crossovers cannot be distinguished since P and N cannot be distinguished where a centromere locus is involved. Similarly, if the center locus, *B*, is the centromere and a four-strand double crossover occurs, it is not possible to tell in which interval it occurred. In this case the A–C pair will be N and one of the other two pairs will be N or P but which is which will be unknown. Any number of loci can be analyzed in this way in groups of three but a complication arises when there are two three-strand double crossovers. With four loci, for instance, if there are three-strand doubles in intervals I & II and in II & III it is useful to know whether the last crossover involved the same strands as the first. In the former case the first and last locus pair will be P, in the latter case N.

FREQUENCIES OF THE CLASSES F, N, AND P

If the number of crossovers occurring simultaneously in an interval is n, and it is assumed that there is no chromatid interference, the proportion of F's between the bounding loci is given by $F = (2/3)[1-(-1/2)^n]$. This formula was derived by MATHER (1935) for the frequency of second division segregation but in the scheme proposed here it is valid for F's between two gene loci as well as between a gene- and a centromere-locus. To proceed further some assumption must be made about chiasma interference. It is convenient to consider two models as reference points, that of no interference in which multiple crossovers occur in a Poisson distribution, and that of complete interference in which only single crossovers occur. If the mean number of crossovers in an interval is m, then with complete interference the frequency of recombinants, $R = m/2$, and $F = m$; hence $R = F/2$. Also $N = 0$. With no interference it is well known that

$$R = (1/2)(1-e^{-m}) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \quad (1)$$

and

$$F = (2/3)(1-e^{-3m/2}) \text{ (PAPAZIAN 1951)} \quad \ldots\ldots\ldots \quad (2)$$

but

$$R = N + F/2$$

so from (1) and (2)

$$N = 1/2 - (1/3)(1-e^{-3m/2}) - e^{-m}/2 \quad \ldots\ldots\ldots\ldots\ldots \quad (3)$$

and since $F + N + P = 1$

$$P = 1/2 - (1/3)(1-e^{-3m/2}) + e^{-m}/2 \quad \ldots\ldots\ldots\ldots\ldots \quad (4)$$

From (2) and (3)

$$2N = 1 - F - (1-3F/2)^{2/3}$$

$$N = F^2/8\,(' + 2F/3) \text{ approximately} \quad \ldots\ldots\ldots\ldots\ldots \quad (5)$$

and

$$R = F/2 + F^2/8\,(1+2F/3) \text{ approximately} \quad \ldots\ldots\ldots \quad (6)$$

Departures from a Poisson distribution of exchanges or from random strand multiple exchanges can be detected by departures from (5) and (6) where only two loci are available. This is valid for long intervals and can be used where other methods of measuring interference cannot. As map distances increase $m \to \infty$ and $F \to 2/3$, $N \to 1/6$, $P \to 1/6$. These limiting values obtain for two independent or loosely linked gene loci one or both of which are far from the centromere. Values of F in excess of 2/3 indicate either an excess of single crossovers or an excess of 3-strand doubles. Values of R in excess of 1/2 always indicate an excess of 4-strand doubles.

THE ADDITION LAW AND THE POSITION OF THE CENTROMERE

Considering recombination frequencies, if the mean number of crossovers, m, is taken to be additive, *i.e.*, $m_{1,2} = m_1 + m_2$, then from (1) (preceding section) $R_{1,2} = R_1 + R_2 - CR_1R_2$ where C varies from 0 with complete interference to 2 with no interference (HALDANE 1919). For F frequencies the corresponding addition law is, from (2) $F_{1,2} = F_1 + F_2 - C_F F_1 F_2$ where C_F varies from 0 to 3/2. In the case of two gene loci on different chromosomes absence of interference may be assumed and since all centromeres undergo first division segregation they can all be regarded, for the present purpose, as a single locus the relationship $F_{1,2} = F_1 + F_2 - (3/2)F_1F_2$ holds. The two chromosomes can be thought of as joined at their centromeres and $F_{1,2}$ the total distance between the two gene loci is the sum of each F distance between gene and centromere. This formula is identical with that given by PERKINS (1949) and WHITEHOUSE (1949) following a different line of argument.

When there are as many as three unlinked loci it is possible, by means of this formula, to map each locus in relation to its centromere (PERKINS 1949; LINDEGREN 1949; WHITEHOUSE 1950). The $F_{1,2}$ values of each of the three pairs of loci are equated to the corresponding F_1 and F_2 values which are unknown and since there are three equations and only three unknowns they can be solved. The solutions are of the form

$$F_1 = 2/3 \pm (2/3)\sqrt{(2-3F_{1,2})(2-3F_{1,3})/2(2-3F_{2,3})}.$$

It is clear that where F_1 has two solutions, their sum will always be 4/3. Not all combinations of alternative solutions will, however, be valid when they are tested in the original equations. It can, in fact, be shown that it is impossible for more than two sets of solutions ever to be valid. The solutions are unreliable for F_1 when $F_{2,3}$ has a value near 2/3.

Even where there are two loci on one chromosome and one on another chromosome the position of the centromeres can be estimated (PAPAZIAN 1951). In this case there will be two equations of the type $F_{1,2} = F_1 + F_2 - (3/2)F_1F_2$ for the pairs of loci on different chromosomes but for the pair of loci on the same chromosome absence of interference cannot be assumed and the constant C_F cannot be assumed to be 2/3; for short distances zero is a better approximation. Where the two linked loci are on different sides of the centromere the third equation is the same as before but where they are on the

same side a subtraction is required and the appropriate equation is $F_{1,2} = F_1 - F_2 + C_F F_1 F_2$.

EVIDENCE FOR INTERFERENCE FROM TETRAD DATA

Good evidence for chromatid interference and for negative chiasma interference in the sex chromosome of *Neurospora crassa* is given by WHITEHOUSE (1942) and LINDEGREN and LINDEGREN (1942) where references to cytological and genetical data bearing on these phenomena in other organisms can be found. In both of these references the same data from LINDEGREN were used in part.

There are a few cases of peculiar tetrad frequencies in the literature which, although open to various objections, deserve attention. WETTSTEIN (1924) describes a cross in the moss *Funaria hygrometrica* with four linked factors. Among 35 tetrads isolated all were two-type although crossing over (2-type non-parentals) was observed in all three intervals. Two-type tetrads were also obtained exclusively under certain conditions by MOEWUS in *Chlamydomonas* (1940), *Brachiomonas* (1940), and *Protosiphon* (1949). In the case of *Chlamydomonas* this was only true at low temperatures; at 22°C 4-type tetrads were obtained. Such results indicate either the regular occurrence of 4-strand double crossovers close together, or of crossing over at the two-strand stage.

In yeast (LINDEGREN 1949), among 83 unordered tetrads the proportion of N's between the loci PN and AD, situated on either side of the centromere, is 0.217. The value of F for the same two loci is 0.53 giving a calculated value of N of 0.042, so that the proportion of N's found is in great excess on an assumption of randomness. It is of interest to examine the apparent anomaly that the total distance between PN and AD is greater than the sum of their separate distances from the centromere. The former is estimated from recombination values and the latter are estimated from segregation values. Following the relations developed in the last section, assuming no interference, the expected value of R can be calculated. The distances of PN and AD from the centromere are 26 and 11 respectively. Therefore $F_1 = 0.52$, $F_2 = 0.22$ and, by the addition law for F's, $F_{1,2} = 0.5695$. From the relation $R = 1/2 + (F^2/8)(1 + 2F/3)$ the theoretical recombination frequency for an F frequency of 0.5695 is 0.33, or 33 map units. The actual map distance between PN and AD was found to be 48. The excess indicates an excess of double crossovers or of 4-strand doubles. The significance of these indications is only tentative owing to the fact that the linkage values of the five gene loci in these crosses are large, the number of asci analyzed relatively small, and the position of the loci therefore uncertain.

The numerous data published by HOULAHAN, BEADLE, and CALHOUN (1949) provide independent evidence on interference in the sex chromosome of *Neurospora crassa*. HOULAHAN *et al.*'s arrangement in table 6 was stated to be the most probable location, but to be based on inadequate data in some cases. Perkins has pointed out to me that crosses 7, 16, 17, and 36 (counting

from the top of table 1) involve genes known with considerable certainty to be in the arm opposite *sex*, whereas the genes in crosses 28, 47, 48, 52, 57, and 66 are less reliably located. Among the four crosses involving the more reliably located genes represented by 139 asci, the number of 2-, 3-, and 4-strand doubles are 2, 1, and 3 respectively. Although there is an excess of 2- and 4- over 3-strand, the numbers are too small to be significant. If the less reliably established loci are added, among 245 asci the numbers are 10, 2, and 4 in which case the excess of 2- and 4- over 3-strand is significant at the one percent level. This compares with a ratio of 20:10:5 (WHITEHOUSE 1942) and 32:14:13 (LINDEGREN and LINDEGREN 1942). In table 2 (HOULAHAN *et al.*) there are 14 crosses (Numbers 1–5, 7–15) involving sex and the albino 4637 which is linked to sex. There are 23 asci (= 0.0827) which are N for these two loci and 51 (= 0.183) that are F. The calculated proportion of F's is 0.00647. This probably indicates both an excess of 4-strand doubles and negative chiasma interference since even with the most favorable, and improbable, chiasma distribution where only double crossovers occur, the N's cannot exceed 1/4 the F's unless there is chromatid interference as well. It is noteworthy that in these crosses there are 22 asci showing double crossovers both in one interval and only one ascus with a double crossover, one in each interval. I am again indebted to D. D. PERKINS for pointing out that 4637 involves a translocation (MCCLINTOCK 1945). This would seem to be connected with the peculiar crossing over pattern but not in any way that is obvious. From similar crosses in table 1 (HOULAHAN *et al.*) the proportions of single crossovers in one arm, single crossovers in the other arm, and double crossovers, one in each arm was analyzed by a new statistical procedure devised by L. J. SAVAGE (appendix II). If genes which are reliably located (numbers 7, 8, 14, 16, 17, 22, and 36) are used, among 197 asci there is no significant excess or deficiency of doubles. If unconfirmed loci (numbers 28, 47, 52, 57, and 66) are added there is an excess of doubles significant at the one percent level. Double crossovers, both in the same arm, are ignored in these calculations. A possible explanation of negative chiasma interference is that crossing over is favored or inhibited in some asci of a perithecium by slight differences in environment or by accidental causes. If this were valid the same negative interference should be found for crossing over in different chromosomes. This was tested from data in table 3 (HOULAHAN *et al.*). Among 912 asci involving loci in different chromosomes there was no significant departure from independence, Again from table 1 (HOULAHAN *et al.*) among 883 asci there was no significant departure from independence of crossing-over between *sex* and the centromere and various other loci and their centromeres on other chromosomes. What departure there was was in the direction expected from a deficiency of simultaneous crossovers or positive interchromosomal interference.

Independent data from the loci sex and 35809 in *N. crassa* (MCDEVITT and BARATT unpublished; BUSS 1944) which lie 5.9 and 9.4 units on either side of the centromere indicate negative chromosome interference across the cen-

tromere. Among 85 asci, pooled from the two sources, there are 10 crossovers in the sex-centromere interval, 16 in the 35809-centromere interval, and four double crossovers, one in each interval. This is about twice the expected number of doubles on a basis of independence of the two events and by exact calculation of a 2×2 table is significant at the one percent level.

Two recent publications give some information as to how an increase or a decrease in total crossing over affects chiasma and chromatid interference. In *N. crassa* data are available (LINDEGREN and LINDEGREN 1942) for crosses made, accidentally, at two different temperatures. The mean frequency of crossovers between the extreme loci sex and pale was about the same at the high (h), and low (l) temperature being 0.404 and 0.390 respectively. At the high temperature the frequency of multiple crossovers was higher, 0.0688 (h) and 0.037 (l), which is significant at the one percent level. Owing to this the frequency of recombinants was actually higher at the low temperature 0.153 (h) and 0.168 (l). This effect was only evident in regions spanning the centromere. Within chromosome arms multiple crossovers were more frequent, but not significantly, at the low temperature 0.012 (l), 0.0089 (h). As regards chromatid interference, at the high temperature the proportion of 3-strand double crossovers was greater relative to the 2-strand and to the 4-strand doubles than at the low temperature, but the 2- and 4-strand doubles were still in excess over random expectation.

In *N. sitophila* it has recently been shown (FINCHAM 1951) that there is more crossing over in the region of the centromere than in *N. crassa*. The data in this paper are interesting and it is to be regretted that the classes of asci have been pooled to such an extent that detailed analysis is impossible. The proportion of F's between the centromere and *crisp* in *N. sitophila* is 0.711 which is in excess of 2/3 and suggests positive chiasma interference or an excess of 3-strand doubles. The proportion of F's between *crisp* and *sex* is 0.666 and the proportion of N's is 0.137 which is not in excess and does not contradict the possibility of an excess of 3-strand doubles or of positive chiasma interference. When F is nearly 2/3 the formula $2N = 1 - F - (3F/2)^{2/3}$ is very unreliable since a small change in F corresponds to a large change in N, the approximate formula given in an earlier section is still more unreliable when F is over 1/2 or so. It would therefore be difficult to tell whether there were a deficiency of N's between crisp and sex.

Independent data (WÜLKER 1935) from *N. sitophila* also indicate that in this species there is no excess of 4-strand doubles, and no negative chiasma interference as found in *N. crassa*, on the contrary, an excess of 3-strand doubles is suggested. The phenotypes of 180 asci analyzed for the factors *sex* and *l/L* (no aerial mycelium) are given in table 4 from which the following were calculated. The frequency of crossovers in the sex arm was 0.422, in the *l/L* arm 0.6, and in both arms simultaneously 0.24 which latter is just under the random value of 0.25. The frequency of F between sex and *l/L* was 0.678 and of N, 0.0833 which is under the expected random value of N

of 1/6. The number of 2-, 3-, and 4-strand doubles is 8, 28, and 8 respectively, this excess of 3-strand doubles is, however, only significant at about the seven percent level.

It would appear from the foregoing as if suppression of crossing over in *N. crassa* relative to *N. sitophila* were accompanied by a considerable relative increase in 2- and 4-strand double crossovers and also in a relative increase of multiple crossovers or negative chiasma interference.

THE SIGNIFICANCE OF CHROMATID INTERFERENCE

The effect of chromatid interference on the usual estimations of map distance and of chiasma interference cannot be treated quantitatively until a measure of chromatid interference is adopted. It is difficult, with present knowledge, to know what sort of measure would be suitable. A simple measure would be one analogous to chiasma interference and would assume that the chances of a particular strand being involved in a second crossover was less or greater than random. This would mean that with positive chromatid interference the departure from randomness would be in the direction 2-< 3-<4-strand and for negative interference 2->3->4-strand. Some of the data, on the other hand, suggest something different, namely that both 2- and 4-strand doubles may be in excess or in deficiency over 3-strand doubles.

The effects of any particular pattern of multiple crossovers can, however, be worked out. One interesting case is that of regular pairs of 4-strand crossovers. If, in such a case, chiasma interference were measured from random spores in the usual way, doubles in adjacent regions would not be detected at all. If the regions under consideration were not adjacent, and one crossover occurred in between, simultaneously with a double, then it would be detected always. Thus chiasma interference would apparently diminish with the distance of the regions concerned. With the usual assumption of chromatid randomness 1/2 of the doubles are detected since the frequency of single crossover strands is 1/2 the frequency of single exchanges and the frequency of double crossover strands is 1/4 that of double exchanges in the tetrad, irrespective of whether the regions are adjacent or not.

The cytological basis for chromatid interference is obscure. Just as chiasma interference can be accounted for, in a vague way, by most theories of crossing over, so chromatid interference can be fitted into many theories. On DARLINGTON's theory it might be due, as suggested by WHITEHOUSE (1942), to two particular strands being more liable to break than the other two. This would not account for an excess or deficiency of both 2- and 4-strand doubles over 3-strand doubles. Among other prominent theories of crossing over that of MATSUURA and HAGA (1942) not only might produce an excess of 2- and 4-strand doubles but would be expected to give a large excess. Furthermore, since the centromeres are post reduced in 2/3 of the cases all genes should show 2/3 post reduction unless a specific pattern of exchange between chromatids from different pairs occurred. On the theory proposed by BELLING

(1933) an excess of 2-strand might be expected. This theory differs from DARLINGTON's in its emphasis on the differentiation of the chromatid into genetic and non-genetic material, each of which duplicate independently. Single crossovers and multiple crossovers involving the same two strands are consequences of the hypothesis that non-genetic material " duplicates " by the formation of new strands independently, as regards position, of the old. Three- and 4-strand doubles involve, in addition, an exchange of strands of a different kind which might occur within the gene, or at the junction of genetic and non-genetic material.

SUMMARY

Two kinds of tetrad, ordered and unordered, are distinguished by the constant spacial position of spores in the former. The interpretation of various spacial patterns in ordered tetrads is described.

Both ordered and unordered tetrads are classified on the basis of the relations of pairs of loci. These relations can be two-type parental (P), two-type non-parental (N), or four-type (F). The relations of various patterns of single and multiple cross-overs to the classes of tetrad are given in tabular form.

On the assumption of no chiasma or chromatid interference, the addition law for F (= 2nd division segregation) frequencies is shown to be $F_{1,2} = F_1 + F_2 - 3F_1F_2/2$; and the relation of recombinant frequencies to F frequencies, $R = 1/2 - [(1 - 3F/2)^{2/3}]/2$.

Evidence bearing on chromatid and chiasma interference is extracted from various recent publications and a convenient statistical method, devised by L. J. SAVAGE, is applied to estimating interference with data pooled from numerous crosses involving small numbers.

APPENDIX I

The type of tetrad with respect to x loci may be represented by a $n \times 4$ rectangular array of 0's and 1's with exactly two zero's in every column. For example:

$$\begin{array}{cccccc} 0 & 1 & 0 & 1 & 0 & 1 \\ 1 & 1 & 0 & 0 & 1 & 0 \\ \hline 1 & 0 & 1 & 0 & 0 & 1 \\ 0 & 0 & 1 & 1 & 1 & 0 \end{array}.$$

Since the two zero's in each column can be chosen in 6 different ways, the total number of types possible, $T(x)$, is, $T(x) = 6^x$.

Ordered tetrads

In an ordered tetrad notice is taken of the way in which cells lie as regards top and bottom two in a linear tetrad of four cells but no notice is taken of which pair are top and which are bottom, or of which single cell is top or bottom in any pair. Two types of tetrad of which one can be obtained from the other by interchanging individual cells of a pair, or by interchanging top with bottom pairs of cells, belong, so to speak, to the same species. The problem is to evaluate the number of species $S(x)$.

The species may be conveniently divided into three genera thus:

Name	Description	No. of types per species in this genus	No. of species in the genus
G_1	w, x; y, z	8	$G_1(x)$
G_2	w, w; x, x	2	$G_2(x)$
G_3	w, x; w, x	4	$G_3(x)$

It is clear that $S(x) = G_1(x) + G_2(x) + G_3(x)$

$$T(x) = 6^x = 8G_1(x) + 2G_2(x) + 4G_3(x).$$

By the same argument which led to $T(x)$, there are 2^x types in G_2 and $2 \times 2^x = 2^{x+1}$ types in G_3, therefore $G_3(x) = (1/2)\ 2^x = 2^{x-1}$

$$G_2(x) = (1/4)\ 2^{x+1} = 2^{x-1}.$$

$$T(x) = 6^x = 8G_1(x) + 2^x + 2^{x-1} = 8G_1(x) + 3 \times 2^x$$

$$G_1(x) = (6^x - 3 \times 2^x)/8$$

$$S(x) = (6^x - 3 \times 2^x + 2^{x+3} + 2^{x+2})/8 = (6^x + 5 \times 2^x)/8$$

This formula was obtained by WHITEHOUSE (1942) empirically but was misprinted.

Unordered tetrads (complete classification)

In an unordered tetrad no notice is taken of the way in which cells lie as regards top two and bottom two and the genus may be divided into two species thus:

Name	Description	No. of types per species in this genus	No. of species in the genus
K_1	w, x, y, z	24	$K_1(x)$
K_2	w, w, x, x,	6	$K_2(x)$

$$S^u(x) = K_1(x) + K_2(x)$$

$$T(x) = 24K_1(x) + 6K_2(x).$$

By the previous argument there are 2^{x-1} types in K_2, therefore,

$$T(x) = 6^x = 24K_1(x) + 3 \times 2^x$$

$$K_1(x) = (6^x - 3 \times 2^x)/24 = (G^{x-1} - 2^{x-1})/4$$

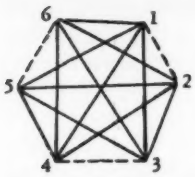
$$S^u(x) = (6^{x-1} - 2^{x-1} + 4 \times 2^{x-1})/4 = (6^{x-1} + 3 \times 2^{x-1})/4.$$

Unordered tetrads (P, N, and F basis)

Any two columns (or loci), say c and d, of the $n \times 4$ matrix representing a tetrad must be in one of the three reflexive and mutually exclusive relations P, N, or F to each other: cPd, if c and d agree in every row; cNd, if c and d disagree in every row; cFd if c and d agree in two rows only.

Two tetrads are said to be of the same family if every pair of loci in one is in the same relation as the corresponding pair in the other. The problem is to compute the number $Q(x)$ of families when there are x loci. The computation of $Q(x)$ is based on certain considerations about the relations P, N, and F.

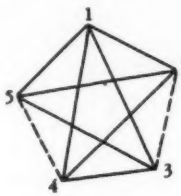
In the first place, if cPd, then to any other locus, say e, c and d must both bear the same relation. Thus P gathers the loci into clusters. What patterns are possible among clusters? Since there are but six different patterns of zeros and ones available, there can be at most six different clusters. If there are actually six they must be represented by some permutation of the pattern below, where solid lines denote F and dotted ones N.



15

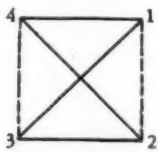
If six clusters are determined, as many possibilities may be formed from them as there are ways to arrange six things in pairs, or $6!/(2!)^3 3! = 15$. The number 15 has, for reference been written beside the figure.

Similarly five different clusters must be in a permutation of the pattern:

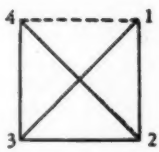


15

For four clusters two different sorts of patterns are possible:

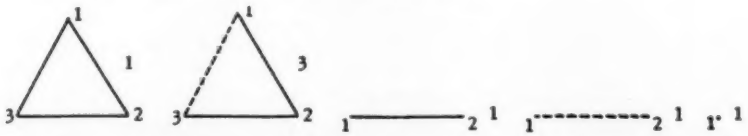


3



6

For three, two, and one the possibilities are these:



It is not a difficult combinatorial problem to see that the number of ways $P(k, x)$ in which x loci can be divided into k clusters is given by

$$k!\,P(k, x) = \sum_{0}^{k} (-1)^{k-j} \binom{k}{j} j^x \qquad (1)$$

If $P(k, x)$ were to be seriously studied for large x, the formula (1) would doubtless be useful. The following recursion formula would also be useful and it provides a convenient basis analogous to Pascal's triangle for computing $P(k, x)$ for small values of k and x.

$$P(k, x) = kP(k, x-1) + P(k-1, n-1)$$
$$Q(x) = P(1, x) + 2P(2, x) + 4P(3, x) + 9P(4, x) + 15P(5, x) + 15P(6, x).$$

The possible number of various kinds of tetrad and of random spores is given for values of x up to five in table 2.

TABLE 2

Numbers of possible tetrads and random spores when different numbers of loci are involved.

Number of loci	1	2	3	4	5
Total kinds of tetrad	6	36	216	1,296	7,776
Ordered tetrads	2	7	32	172	4,860
Unordered tetrads (complete)	1	3	12	60	336
Unordered tetrads (P, N, F, basis)	1	3	11	48	236
Random spores (2^x)	2	4	8	16	32

APPENDIX II

The statistical problem here is this. Several double dichotomies are observed, each constituted by a small number of observations. A statistical test is sought for the hypothesis that each is positively correlated.

Tradition would suggest that the hypothesis be tested by computing χ^2 for each double dichotomy, adding the values of χ^2 and appealing to the χ^2 test. This solution is rather unsatisfactory in two respects both of which may be met by a variation of it.

First, since each double dichotomy is small, the expectations of the individual hypotheses will, under the null hypothesis, each be a little less than one, so that the χ^2 test based on this sum will be systematically inaccurate. Since $\chi^2 = t^2(n-1)/n$, it may be noted that, as was asserted earlier $E(\chi^2) = (1-1/n)$ $E(t^2) = (1-1/n) < 1$.

Second, even if the double dichotomies were not small, adding the individual values of χ^2 leads to a test which is, at least to all appearances, less sensitive than a test based on the sums of χ itself would be.

To construct the test we propose, let a double dichotomy be represented by the familiar table.

a	b	y
c	d	z
w	x	n

where $w = a + c$, $n = w + x$, etc., and let $\Delta = ad - bc = an - yw$, and compute its expected value and variance of Δ under the null hypothesis thus:

$$E(\Delta) = nE(a) - yw = 0$$
$$V(\Delta) = n^2V(a) = wxyz/n - 1,$$

the expected value and variance of a is given in any account of the hypergeometric distribution. The quantity $t = \Delta\sqrt{(n-1)/wxyz}$ therefore has, under the null hypothesis, zero expectation and unit variance, provided that $wxyz > 0$. The sum of several values of it should therefore, under the null hypothesis, be approximately normally distributed about zero, with variance equal to the numbers of terms in the sum. Under the envisaged alternatives it will tend to be positive.

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THE EFFECT OF INBREEDING ON THE VARIATION DUE TO RECESSIVE GENES *

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IN his classical treatment of inbreeding, WRIGHT (1921) developed the concept of the inbreeding coefficient F, which he defined as the correlation between the genetic constitution of the gametes in the uniting egg and sperm. This is directly related to the heterozygosity remaining in the population which is equal to $1 - F$ times the heterozygosity at the start of inbreeding. If a number of inbred lines are made without selection from a random breeding population, the genetic variance due to genes which act additively increases between lines as $2F$ and decreases within lines as $1 - F$. (If inbreeding is rapid, the value $1 - F$ for the genetic variance within lines is not adequate, the correct expression being $1 + \bar{F} - 2\bar{F}$, where $\bar{F}$ is the inbreeding coefficient of the hypothetical progeny produced by random mating within lines in the present generation and $2\bar{F}$ is correct for the variance between lines.) For genes which do not act additively, there is not the same correspondence between heterozygosity and variance and the above relationships do not hold. As we know little about the dominance relationships of the genes controlling continuous variation, it seemed desirable to investigate theoretically the effect of inbreeding on the variation due to genes which are completely recessive and to genes which show overdominance. Particular attention is given to the case in which the recessive (or quasi-recessive) is at low frequency as this is the most probable situation in natural populations.

We shall deal first with continued full-sib mating in which the results can be worked out by simple, if rather laborious, arithmetic and where the process can be most easily visualised. The more general situation of slow inbreeding in lines of constant breeding size requires more sophisticated mathematics and the details of the derivations are given in an appendix. The two methods are in good agreement.

CONTINUED FULL-SIB MATING

This system of mating can be treated most simply by the method of mating types, originally used by JENNINGS (1916) and subsequently with the help of matrix theory by HALDANE (1937) and by FISHER (1949). If only two alleles, A and a, are present in the population at a locus, there are six possible types of matings. The relative frequencies of matings of different types in any generation can be calculated from the frequencies in the previous generation

* Part of the cost of the accompanying mathematical formulae has been paid by the GALTON and MENDEL MEMORIAL FUND.

on the assumption that the offspring are mated at random. For instance, matings Aa × aa will give offspring ½ Aa, ½ aa and if these are mated at random, one quarter of the matings will be of the type Aa × Aa, one half Aa × aa and one quarter aa × aa. The equations giving the frequencies of the six types in terms of the frequencies in the preceding generation are shown schematically in table 1 in which y_0, for instance, signifies the frequency of AA × Aa matings in the zero generation.

Reading horizontally, we have, for instance, $u_1 = \frac{1}{4} y_0 + z_0 + \frac{1}{4} u_0 + \frac{1}{4} v_0$. If a is completely recessive to A and the phenotypic value of AA, Aa is taken as zero and of aa as unity, the genetic variance within the progeny of Aa × Aa and Aa × aa matings is 3/16 and 1/4 respectively, the other mating types having no variation within their progeny. (Throughout the paper, "genetic variance" will be used in the sense of all variance due to gene segregation.) The average variance within lines (each line being in this case a single mating) is 3/16 u + 1/4 v. The genetic variance between lines is easily calculated, as the expected value for the progeny is zero for all types except those represented by u, v, and w for which it is ¼, ½ and 1 respectively.

TABLE 1

Frequencies of the various mating types.

	AA × AA x_0	AA × Aa y_0	AA × aa z_0	Aa × Aa u_0	Aa × aa v_0	aa × aa w_0
x_1	1	¼		1/16		
y_1		½		¼		
z_1				⅛		
u_1		¼	1	¼	¼	
v_1				¼	½	
w_1				1/16	¼	1

In the computations, we take as x_0, y_0, z_0, etc. the values for a random-bred population and evaluate the set x_1, y_1, z_1, etc., and so on. Figure 1 shows the frequencies of mating types other than AA × AA for the case when $q_a = 0.1$. The immediate effect of the inbreeding is to cause y to decrease and u and v to increase. After about 6 generations, the frequencies of all types of matings except AA × AA and aa × aa become practically constant relative to one another and then decline to zero when inbreeding is complete. At that point, x = 0.90 and w = 0.10, as all lines become homozygous for either A or a. The variance within lines increases considerably in the first generation (F = 0.25) to 2.99 times its random breeding value, remains fairly stationary for two further generations, and then declines. The variance between lines increases continually as the inbreeding progresses, the rise being almost linear for the first six generations (fig. 2). Consideration of the first generation only shows that the variance within lines will only increase above its random breeding value if the frequency, q, of the recessive gene is below 0.47. At low gene frequencies, at the start almost all the a genes will be carried in matings AA × Aa which will have frequency approximately 4q, the frequencies of other matings in which heterozygotes take part being in higher powers of q. Thus

all the matings, except those of type $AA \times AA$, will derive from an initial entry of y_0 as 4q. It follows that the shape of the curves showing the effect of inbreeding on variation will become independent of q as q decreases, as all entries will be multiples of q. Computation shows that in this case, the variance within matings reaches its maximum value of 0.207q after three generations of inbreeding ($F = 0.50$). Under random mating conditions, matings of type $Aa \times Aa$ will make the major contribution to the variance within mat-

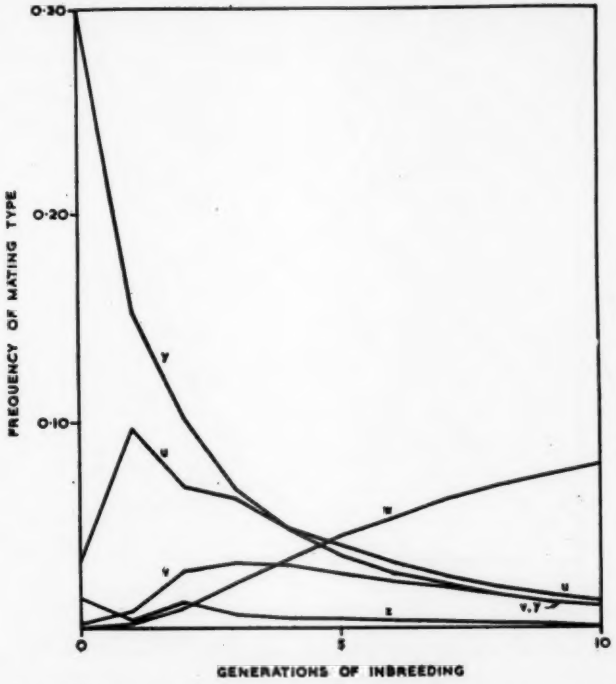

FIGURE 1.—Frequencies of various mating types (except $AA \times AA$) with continued full-sib mating. The mating types are designated as: y ($AA \times Aa$), z ($AA \times aa$), u ($Aa \times Aa$), v ($Aa \times aa$), and w ($aa \times aa$).

ings with frequency $4q^2(1-q)^2 \sim 4q^2$ and variance 3/16, giving an average variance of $\frac{3}{4}\ q^2$. At its maximum, the variance within matings is therefore $\frac{0.207q}{0.75q^2} = \frac{0.276}{q}$ times its random breeding value. Figure 3 shows the curves for the different variances when q is very low, which are not very different from those in figure 2 for $q = 0.10$.

The total genetic variance rises continuously as the inbreeding progresses. When complete homozygosis is reached, the total variance is $q(1-q)$, q lines having phenotypic value unity and $1-q$ having value zero. With random mating, when a fraction q^2 have genotype aa and phenotype value one and

the rest have value zero, the total variance is $q^2(1-q^2)$. Of this, the additively genetic component, the portion usually detected in genetic analyses such as heritability studies, is only $2q^3(1-q)$. When inbreeding is complete, the total variance is equal to $1/q(1-q)$ times its random breeding value and $1/2\,q^2$ times the part of the variation that can usually be detected in the absence of inbreeding. For genes which act strictly additively, the total variance at com-

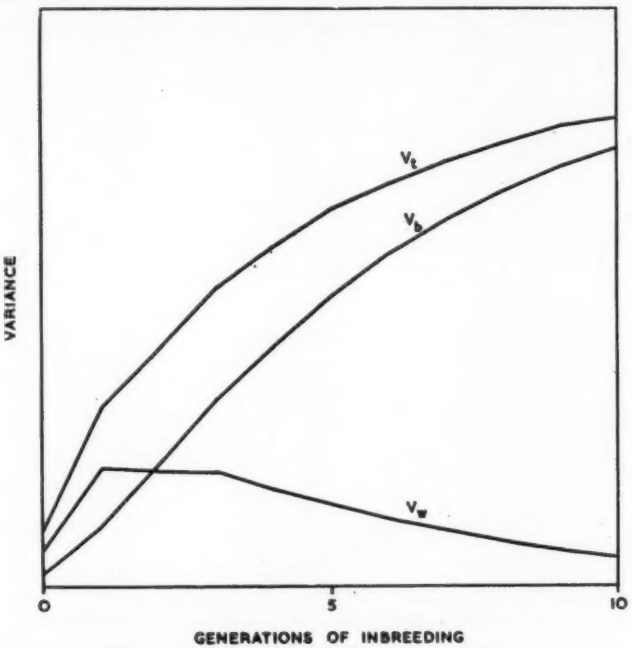

FIGURE 2.—Total variance (V_t), variance within lines (V_w), and variance between lines (V_b) with continued full-sib mating and $q_0 = 0.1$.

plete homozygosis is twice the total variance under random breeding conditions, the latter being, of course, equal to the additive component.

INBREEDING IN LINES OF CONSTANT BREEDING SIZE

In the more general case with slow inbreeding, in which each line is of constant breeding size, it is more convenient to consider the inbreeding from the point of view of the change of gene frequency in the several lines. Starting from a population with a given gene frequency, the frequencies in the lines, as inbreeding progresses, gradually scatter further and further from the original value due to sampling (the phenomenon which in discussions of evolution is often called the " Sewall Wright drift "). The frequency averaged over the whole population of lines remains the same as that in the original

random breeding population, if there has been no selection. When inbreeding is complete, the gene frequency in each line is either 0 or 1. The variance within and between lines can then be related to the distribution of the gene frequency in the several lines. Within a line in which the gene frequency is q_1, the genetic variance is $q_1^2(1-q_1^2)$ so that the average value of the genetic variance within lines is $\mu_2 - \mu_4$, where the μ's are the moments of the q distribution about zero. By a similar argument, the genetic variance between lines

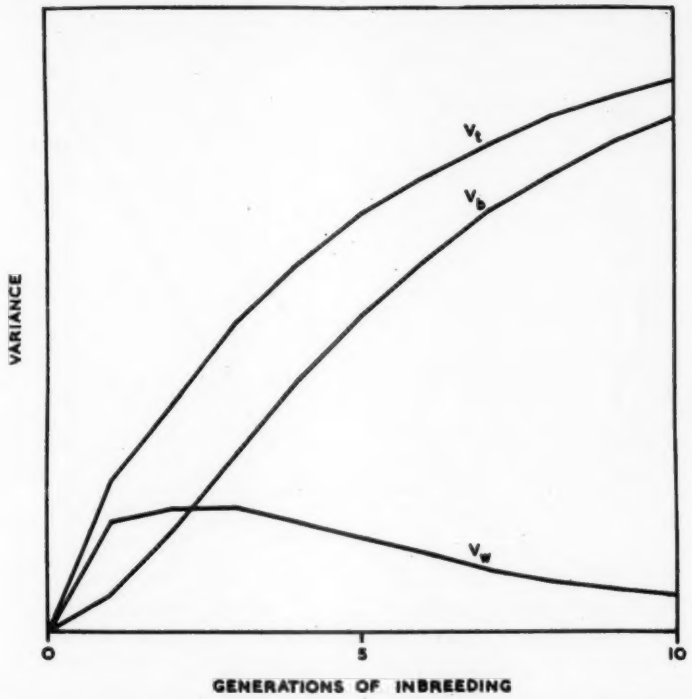

FIGURE 3.—Total variance (V_t), variance within lines (V_w), and variance between lines (V_b) with continued full-sib mating and q_0 very small.

is the variance of q_1^2, $\mu_4 - \mu_2^2$ and the total genetic variance is $\mu_2 - \mu_2^2$. The evaluation of general expressions for the moments, and therefore of the variances, as inbreeding progresses, depends on matrix theory and is given in an appendix. The within line variance is given by

$$V_w = a(1-F) + b(1-F)^3 + c(1-F)^6$$

where $a = 0.8\,q(1-q)$

$b = -q(1-q)(1-2q)$

$c = 0.2q(1-q) - q^2(1-q)^2$.

When $F = 0$, $V_w = a + b + c = q^2(1 - q^2)$ and when $F = 1$, $V_w = 0$. The change of V_w when F is small can be judged from the coefficient of F in the expansion of the above equation, which on simplification becomes $q(1 - q)\ (1 - 6q^2)$. V_w will then only pass through a maximum if $1 - 6q^2 > 0$, *i.e.*, $q < 0.41$ and will otherwise decline continuously. As F increases, the last two terms in V_w decrease in importance until V_w becomes proportional to $1 - F$. When q is small, V_w becomes $q\ [0.8(1 - F) - (1 - F)^3 + 0.2(1 - F)^6]$, and rises to a

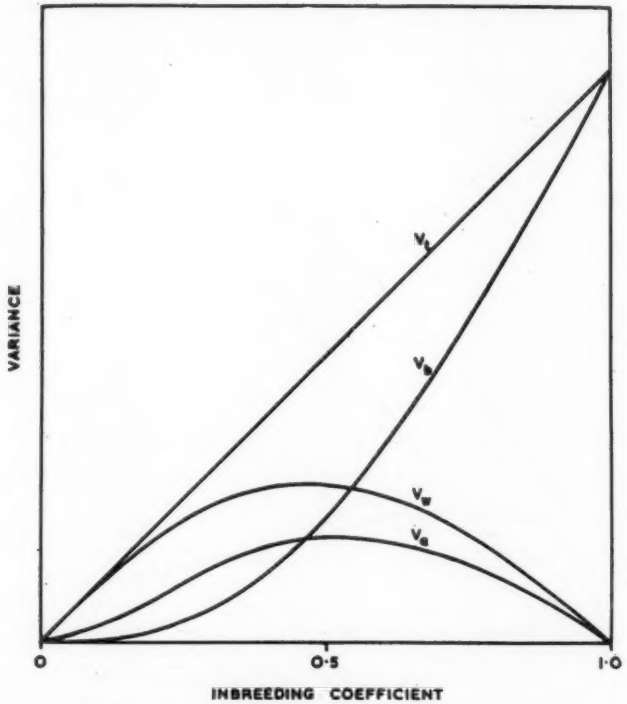

FIGURE 4.—Additive variance within lines (V_a), total variance (V_t), variance within lines (V_w) and variance between lines (V_b) with random mating in a population of constant size with q_0 very small.

maximum of $0.280q$, when $F = 0.46$, compared to $q^2(1 - q^2)$ in the random bred population. The maximum value is then roughly $\frac{0.280}{q}$ times the random-breeding value, in good agreement with the value of $\frac{0.276}{q}$ obtained from continual full-sib mating.

The additive component of the variance within lines, the component detectable by such techniques as parent-offspring regression, is for a given line

$2q_1^3(1-q_1)$ so that the average value of this, V_a, is $2(\mu_3-\mu_4)$. In the above terminology this is given by $.75a(1-F)+b(1-F)^3+2c(1-F)^6$.

It is not possible to give simple formulae for the between line or total variance except when q is small. Then, expanding V_b as a power of F, the term with the lowest power of y is $3F^3q$. The total variance is then Fq. Figures 4 and 5 show the behaviour of V_t, V_b, and V_w when q is very small and when $q = 0.10$. The curves show clearly the main features of the effect of inbreeding on the variance. V_w and V_a increase to a maximum when $F = 0.4$

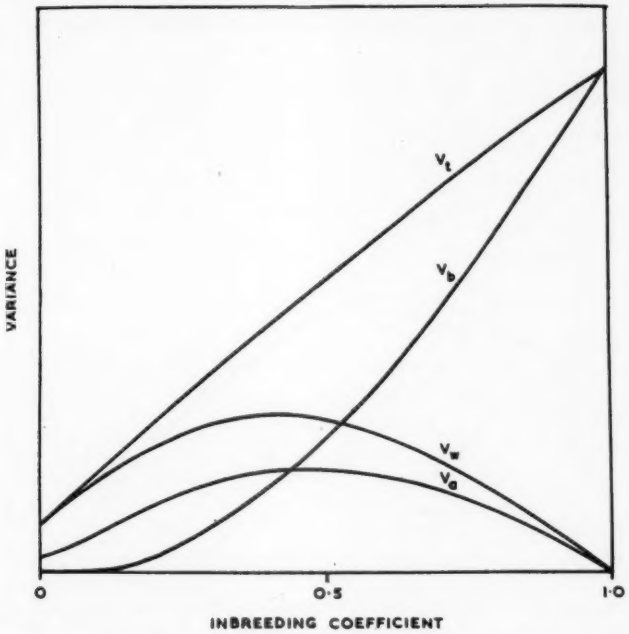

FIGURE 5.—Additive variance within lines (V_a), total variance (V_t), variance within lines (V_w) and variance between lines (V_b) with random mating in a population of constant size with $q_0 = 0.1$.

to 0.5 and then decline. For small values of q, V_a increases as F^2 when F is low. As F approaches unity, V_a becomes equal to three-fourths of V_w. V_b increases slowly at the start as F^3 but increases more rapidly when F is greater than 0.50. V_t increases almost linearly with F in both cases.

Some discrepancies will be noted between the results obtained from the two models. For instance, in the continuous case there is no variation between lines at zero inbreeding whereas there is in the full-sib case. The continuous case, deriving from random mating between a chosen number of parents, automatically includes the possibility of some self-fertilization. The first generation to show variation between lines is therefore inbred. If self-fer-

tilization is explicitly excluded, as of course in a bisexual organism, it is possible to have variation between lines (or rather between families) without the animals being inbred. However, the two systems are otherwise in good agreement.

GENES SHOWING OVERDOMINANCE

It is possible that, at some loci, the phenotypic value of the heterozygote may lie outside the range of those of the homozygotes. Of the extent and kind of such " overdominance " we know very little. As a model, it will be assumed

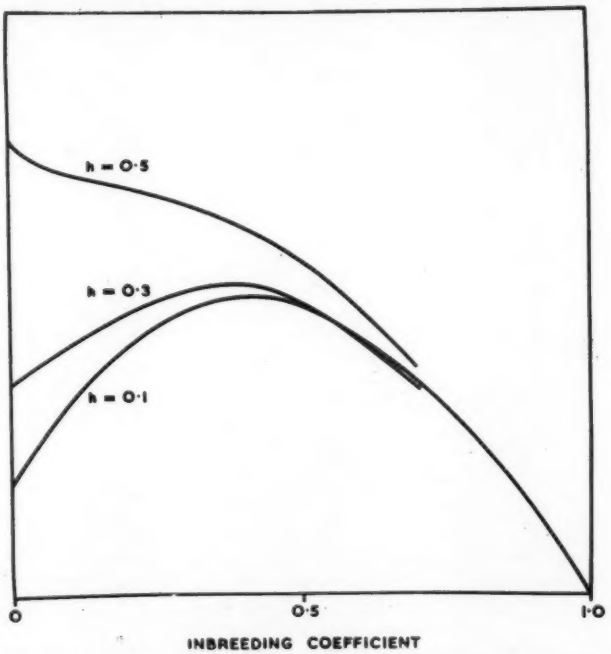

FIGURE 6.—Variance within lines for a locus with overdominance. Phenotypic values are AA = h, Aa = 0, aa = 1 and $q_0 = 0.1$.

that the phenotypic values are AA = h, Aa = 0 and aa = 1. Using the moment terminology, the variance between lines is then given by

$$V_w = 2h^2\mu_1 + (1 - 2h - 5h^2)\mu_2 + 4h\,(1 + h)\mu_3 - (1 + h)^2\mu_4.$$

Figures 6 and 7 show the within and between line variances for several values of h when q = 0.10. The curves for h = 0.1 are little different from those for h = 0 in figure 4 but as h increases, the increase of V_w with F becomes less until for a value of h slightly over 0.3, V_w decreases continuously with F. From these results, it seems likely that the general conclusions

arrived at for completely recessive genes will also apply to genes showing overdominance provided h is less than 0.2.

THE EFFECTS OF SELECTION

In the earlier analysis it was assumed that there was no selection against the recessive gene. As, in general, recessives cause some decline in fitness when they are homozygous, it seemed worthwhile to calculate the changes in variance in the extreme case when the selection against the homozygous recessive is complete. Here, the mean frequency of the recessive gene in the

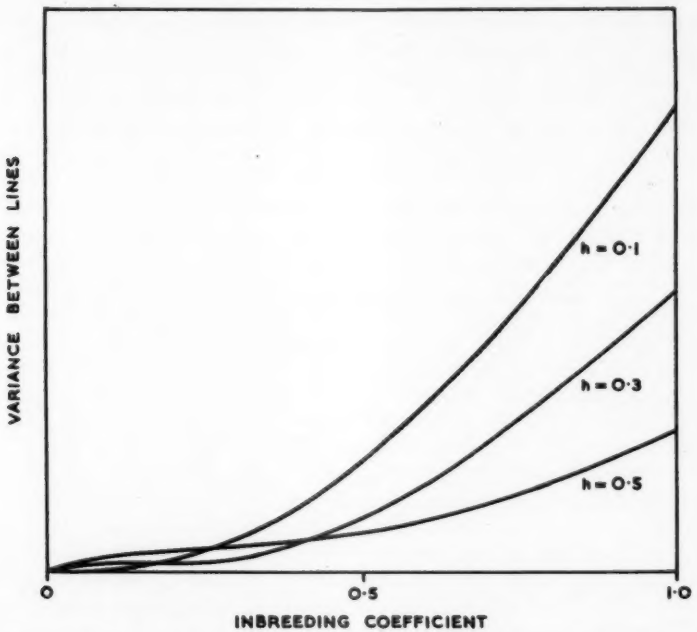

FIGURE 7.—Variance between lines for a locus with overdominance. Phenotypic values are AA = h, Aa = 0, aa = 1 and $q_0 = 0.1$.

population of lines will not remain the same but will gradually decline as selection proceeds. The genetic variance will not depend on the inbreeding coefficient alone but also on the amount of selection and therefore on the number of generations that the inbreeding and selection has proceeded. There will thus be no general solution in terms of F and each inbreeding system will have to be treated separately. For continued full-sib matings, when selection against aa animals is on an individual basis, there are only three possible types of mating as shown in table 2.

The average variance within lines is $3u/16$ and that between lines is $u(1-u)/16$. The results obtained are shown in figure 8 for $q = 0.10$ and

TABLE 2

Frequencies of the various mating types with complete selection against the recessive factor.

	AA × AA x_0	AA × Aa y_0	Aa × Aa u_0
x_1	1	1/4	1/8
y_1		1/2	4/8
u_1		1/4	4/8

the curve for V_w from figure 2 is included for comparison. Both V_b and V_w rise at first and then decline to zero as inbreeding approaches completion and all lines become AA in constitution. In the early stages, the effect of selection on the behaviour of the variance within lines is fairly small. V_w rises to 2.69 times its random-breeding value in the first generation and does not decline below the random-breeding value for 7 generations. With no selection, the maximum V_w is 2.99 times the random-breeding value and it takes 10 generations to decline to that value again. The selection against the recessive on this model is the most stringent possible on an individual basis and one can safely make the generalization that in the early generations of full-sib

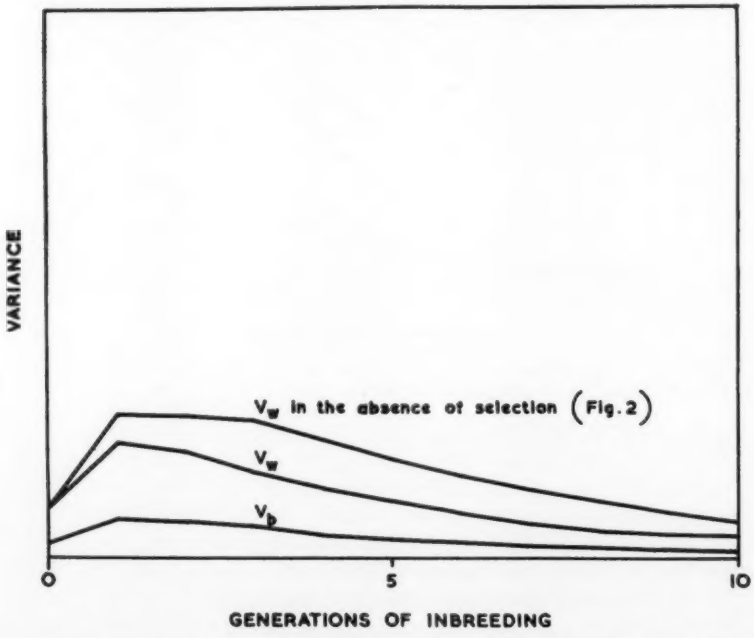

FIGURE 8.—Variance within lines (V_w), and variance between lines (V_b) with full-sib mating and complete selection against the recessive allele, whose initial frequency was 0.1.

mating, selection will not greatly affect the behaviour of the variance within lines. If the inbreeding proceeds more slowly, selection will be more important as it will have more opportunity to take effect.

THE PERFORMANCE OF THE LINES IN CROSSING

From the practical point of view more interest attaches to the performance of the crosses between lines than to that of the lines themselves. The performance of the crosses made between members of a group of lines is often discussed in terms of " general combining ability " and " special combining ability." The " general combining ability " of a line refers to the average performance of the crosses between that line and all the other lines. The " special combining ability " of a particular cross refers to the difference between the performance of the cross and what would have been expected from the general combining abilities of the parent lines. In mathematical terms, the performance of a particular cross P_{ij} between the i^{th} and j^{th} lines is given by

$$P_{ij} = m + a_i + a_j + a_{ij}$$

where m is the mean of all crosses, a_i, a_j are the general combining abilities of the i^{th} and j^{th} lines and a_{ij} is the interaction term, the special combining ability. The term " top-cross " refers to the crosses made between a line and a sample of individuals from the random-bred population. The average top-crossing performance of a line should be equal to the general combining ability in crosses with lines drawn without selection from the random-bred population, because the gametes from a group of inbred lines made without selection are exactly equivalent to a random sample of gametes from the random-bred population. In a similar manner, a series of crosses made at random between completely inbred lines made without selection are equivalent to a group of individuals drawn from the random-bred population.

Consider two lines in which the gene frequencies of the recessive are q_1, q_2. Then, assuming complete dominance, the average performance of the cross between them is $q_1 q_2$. The general combining ability of a line with gene frequency q_1 is $q_1 q$ where q is the average gene frequency in the lines and in the original population. The variance between lines in general combining ability is q^2 var $q_1 = Fq^3(1-q)$. The variance between crosses is var $(q_1 q_2)$ where q_1, q_2 are independent samples from a known distribution. This can be evaluated from the moments about zero of the distribution as $\mu_2^2 - \mu_1^4$ and is equal to $q^2(1-q)F(F+2q-Fq)$, being equal to the variance in the random-bred population, $q^2(1-q^2)$, when $F = 1$. From the above equation for P_{ij} the variance between crosses due to special combining ability is equal to the total variance between crosses minus twice the variance between lines in general combining ability. This equals $F^2 q^2(1-q)^2$. When F is small compared to q, therefore, the variance between crosses is mostly due to general combining ability but as F increases, the special combining ability becomes much more important. Figure 9 shows the relative contributions that the two parts make to the variance between crosses when $q = 0.10$. From the practical as-

pect, it is the best cross between members of a group of lines that is important. For a given number of lines, the probable superiority of the best cross above the mean will be proportional to the standard deviation between crosses and will be proportional to the first power of F if q is small.

The correlation between the performance of lines in crossing when the lines are partially inbred with the performance when inbreeding is complete is of some practical interest. As inbreeding causes the gene frequencies to deviate between lines but does not change the average gene frequency in the

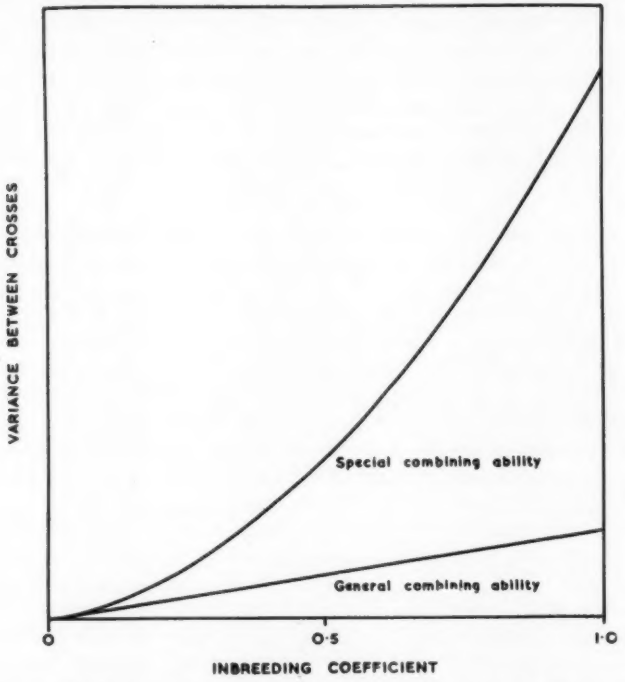

FIGURE 9.—Relative contribution of general and specific combining ability to the variance between crosses with change in inbreeding. $q = 0.1$.

whole population of lines, it follows that the expected gene frequency in completely inbred lines deriving from a given partial inbred line is equal to the gene frequency in that line. Taking the cross between two partially inbred lines in which the frequency of the recessive is q_1 and q_2, we want to know the expected value of $q_1 q_2$ when the inbreeding is complete. Because inbreeding does not change the expected gene frequency, it also does not change the expected value of the cross between two lines in the absence of environmental variation or errors of measurement. In other words, the regression of the

future performance of a cross between two lines on its present performance will always be unity, irrespective of the stage at which the lines are measured. From this, it follows that the correlation between future performance and present performance will be equal to

$$\sqrt{\frac{\text{variance of present performance}}{\text{variance of future performance}}}.$$

In particular, for general combining ability, this equals

$$\sqrt{\frac{Fq^2(1-q)}{q^2(1-q)}} = \sqrt{F}$$

and for the performance of a specific line cross it is

$$\sqrt{\frac{q^2(1-q)\,F(F+2q-Fq)}{q^2(1-q^2)}} = \sqrt{\frac{F(F+2q-Fq)}{1+q}}$$

which is equal to F when q is small.

The correlation between the phenotypic value of a line and its general combining ability will be generally for a single gene fairly close to one, being a correlation between q_1 and q_1^2.

VARIATION DUE TO MANY RECESSIVE GENES

We have been dealing above with the variation due to a single recessive gene. In practice, the genetic variation may be expected to be due to many genes with different gene frequencies and effects of different magnitude. Fortunately, this does not greatly complicate the picture and many of the results can be taken over directly from the single gene case. The resultant variance will be merely the sum of the variance due to the separate genes, so that a generalization can be made about the variation due to recessive genes at frequencies less than about 0.3, that the within line variance will increase until F is in the region of 0.5 and then decline and that the between line variance will increase at first as F^3. Indeed, the presence of variation due to many genes means that as far as the within line variance is concerned, lines will deviate less from the predicted behaviour than they would if the variation were due only to a single gene. In a similar way, the formulae referring to the crossing performance of lines for genes of low frequency can also be taken over to the general case as can those for the correlations and regression of future performance and present performance of crosses.

DISCUSSION

The actual experimental evidence on the effect of inbreeding on the variation within lines is fairly scanty but, in general, the decline in phenotypic variation is slight and in some cases it is known to have increased above the original value after several generations of brother-sister matings (*e.g.*, PEASE

1948). Apart from the possibilities arising from the present paper, there are three other possible causes for such a phenomenon.

(a) Natural selection for heterozygotes may be opposing the trend towards homozygosis produced by inbreeding.

(b) In many characters, the greater part of the variation is environmental in origin and therefore will not be affected by inbreeding. In characters like egg production index in poultry or litter size in swine, the changes in genetic variance may be undetectable against the background of the environmental variance.

(c) The inbred lines may differ from the random-bred stock in their response to environmental changes. WRIGHT (1935) has described a line of guinea-pigs in which a proportion of animals are otocephalic. There is considerable variation in head shape within the line which, on testing, was found to be not genetic in origin. It seems that the line has shifted towards some critical threshold in the process of head formation over which a proportion of the environmental variations takes the animals in the course of development, resulting in a variety of different abnormalities of head shape.

To these three factors affecting the total variation within lines, we may now add a fourth—that the variation due to recessive genes at low frequency will increase with inbreeding until F is about 0.50 and may not return to its original value until F reaches close to 1.

We are still fairly ignorant about the exact behaviour of the genes responsible for continuous variation. In some characters, *e.g.*, fat percentage in milk in cattle, it is likely that the genes are acting mostly in an additive manner. In other, in particular, characters with low heritability that show inbreeding depression, *e.g.*, egg production index in poultry, yield in maize, a high proportion of the genetic variation might be due to recessive or overdominant genes. Such genes will generally be held at a low frequency in the population by natural selection. The possible increase of the genetic variance due to such genes with increasing inbreeding has therefore some practical importance. There are some writers who maintain that animals whose performance is inferior are so because they are homozygous for deleterious recessives. They argue that the only way to improve the general level of the stock is to uncover the recessives by inbreeding and so to produce a population with a uniformly high level of performance. In fact, even with stringent selection against such recessives, it will take several generations of brother-sister matings in which the recessives are segregating out before the genetic variance within such an inbred population will decline to its original value. This is only one of several objections to such a programme.

The results presented here may be of some use in providing a possible explanation for some peculiar experimental results but it is doubtful whether they can be of any precise value in the analysis of continuous variation. When the variation is due to several recessive genes at different frequencies, this treatment can only supply a general description of the probable behaviour of the variances, not of sufficient precision for the experimental results to be used to give much information about the behaviour of the genes themselves.

The only situation in which the gene frequencies are known accurately—in a cross between two inbred lines—the position is complicated by linkage. In discussing the variation due to several genes above, it has been assumed that in the initial random-bred population there is no correlation between the genes present at adjacent loci in a gamete. In the F_2 of a cross between two inbred lines, there will be such a correlation between genes at adjacent loci and any analysis will tell us about the properties of such blocks of genes rather than of the individual genes. In the absence of overdominance at individual loci, such blocks of genes will tend to show overdominance themselves, due to the usual covering-up of recessives. It seems therefore that unfortunately the use of such a cross cannot tell us much about the dominance relationships of the individual genes.

SUMMARY

The effect of inbreeding on the variation due to recessive genes has been treated theoretically both for the case of continued full-sib mating and in lines of small breeding size. If the recessives are at low frequency, the variation within lines increases to a maximum when F is close to 0.50, and declines to zero when inbreeding is complete. The additive component of the variance within lines behaves in a similar manner. The variance between lines is small at first, increasing as F^3 when F is small. The total variance in the population of lines increases almost linearly with F. The variance in the performance of crosses between lines is made up of a component due to the general combining ability of lines proportional to F and to a component ascribable to the special combining ability in particular crosses proportional to F^2. The special combining ability thus becomes much more important as inbreeding progresses. The effects of overdominance and selection are also briefly treated.

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APPENDIX

A GENERAL DERIVATION OF THE RELATIONSHIP OF THE GENETIC VARIANCE TO THE COEFFICIENT OF INBREEDING, F

In terms of gene frequency, the effect of inbreeding can be looked upon as the gradual widening of the distribution of gene frequencies until, when inbreeding is complete, q may only take the values 0 or 1. Starting with a known gene frequency, q, in lines of constant breeding size of n/2 animals, we can consider the second generation as derived from the first by the sampling of groups of n haploid sets, the gene frequency in the different

groups being distributed binomially with mean nq and index n. The next generation is then the repetition of this process, each line giving rise to a group of lines whose gene frequencies are binomially distributed about the mean of the parent line. If the number of lines is constant, the sample of existing lines can be considered as a random sample from the above hypothetical population.

Consider, in the r^{th} generation, the lines (having frequency f_1) in which the gene frequency is q_1. These lines will then by the above operation give a new group of lines in which the moments about zero of the gene frequencies are by the usual formulae for a binomial distribution.

$$[\mu_1]_1 = q_1$$

$$[\mu_2]_1 = \frac{q_1}{n} + \left(1 - \frac{1}{n}\right)q_1^2$$

$$[\mu_3]_1 = \frac{q_1}{n^2} + \frac{3}{n}\left(1 - \frac{1}{n}\right)q_1^2 + \left(1 - \frac{1}{n}\right)\left(1 - \frac{2}{n}\right)q_1^3$$

$$[\mu_4]_1 = \frac{q_1}{n^3} + \frac{7}{n^2}\left(1 - \frac{1}{n}\right)q_1^2 + \frac{6}{n}\left(1 - \frac{1}{n}\right)\left(1 - \frac{2}{n}\right)q_1^3 + \left(1 - \frac{1}{n}\right)\left(1 - \frac{2}{n}\right)\left(1 - \frac{3}{n}\right)q_1^4$$

The moments of the total population of lines will be the sum of the moments of the groups of lines, arising from each value of q_1 with appropriate weights f_1.

$$\text{Thus: } {}_{r+1}\mu_2 = \sum_q \frac{{}_rf_1\, q_1}{n} + \sum_q \left(1 - \frac{1}{n}\right){}_rf_1\, q_1^2$$

$$= \frac{1}{n}\,{}_r\mu_1 + \left(1 - \frac{1}{n}\right){}_r\mu_2 \text{ (the } r \text{ subscripts referring to generations.)}$$

Similarly we have three other equations relating the moments in the $(r+1)^{th}$ generation to those in the r^{th} generation, which can be written diagrammatically as follows:

	${}_r\mu_1$	${}_r\mu_2$	${}_r\mu_3$	${}_r\mu_4$
${}_{r+1}\mu_1$	1			
${}_{r+1}\mu_2$	$\frac{1}{n}$	$1 - \frac{1}{n}$		
${}_{r+1}\mu_3$	$\frac{1}{n^2}$	$\frac{3}{n}\left(1 - \frac{1}{n}\right)$	$\left(1 - \frac{1}{n}\right)\left(1 - \frac{2}{n}\right)$	
${}_{r+1}\mu_4$	$\frac{1}{n^3}$	$\frac{7}{n^2}\left(1 - \frac{1}{n}\right)$	$\frac{6}{n}\left(1 - \frac{1}{n}\right)\left(1 - \frac{2}{n}\right)$	$\left(1 - \frac{1}{n}\right)\left(1 - \frac{2}{n}\right)\left(1 - \frac{3}{n}\right)$

Thus, knowing the values in the zero generation, we could work out the values in any generation. By the use of matrix theory, it is possible to obtain a general expression for the moments in any generation. For a four-rank matrix such as the above, there are four latent roots, λ_0, λ_1, λ_2, λ_3, and to each latent root there corresponds a latent vector t (a linear function of the moments) such that:

$$\begin{aligned} {}_{r+1}t_0 &= \lambda_0\, {}_rt_0 \\ {}_{r+1}t_1 &= \lambda_1\, {}_rt_1 \\ {}_{r+1}t_2 &= \lambda_2\, {}_rt_2 \\ {}_{r+1}t_3 &= \lambda_3\, {}_rt_3 \end{aligned}$$

Knowing the zero value for the latent vectors, t, we can easily calculate the values in the r^{th} generation as ${}_rt_0 = {}_0t_0\,\lambda_0^r$ and so on, and therefore also calculate the moments by expressing them as functions of the t's. As the elements to the right-hand of the diagonal are zero, the four latent roots are simply the entries in the main diagonal. As an example of the evaluation of the latent vectors, consider the vector t_2 corresponding to the root λ_2. We may express t_2 as $a_1\mu_1 + a_2\mu_2 + a_3\mu_3 + a_4\mu_4$. We then obtain the coefficients by equating the coefficients of the μ's deriving from the t recurrence equations with those deriving from the μ recurrence equations. Writing $1 - \frac{1}{n} = \lambda_1$, etc. we have

$$a_4\lambda_2 = a_4\lambda_3$$

$$a_3\lambda_2 = a_4\frac{6\lambda_2}{n} + a_3\lambda_2$$

$$a_2\lambda_2 = a_4\frac{7\lambda_1}{n^2} + a_3\frac{3\lambda_1}{n} + a_2\lambda_1$$

$$a_1\lambda_2 = a_4\frac{\lambda_0}{n^3} + a_3\frac{\lambda_0}{n^2} + a_2\frac{\lambda_0}{n} + a_1\lambda_0$$

giving

$$a_4 = 0$$

$$a_3 = 1 \text{ (an arbitrary value)}$$

$$a_2 = -\frac{3}{2}$$

$$a_1 = \frac{1}{2}$$

Then $$t_2 = \frac{1}{2}\mu_1 - \frac{3}{2}\mu_2 + \mu_3$$

The four equations for the t's are then:

$$\lambda_0 = 1 \qquad t_0 = \mu_1$$

$$\lambda_1 = 1 - \frac{1}{n} \qquad t_1 = -\mu_1 + \mu_2$$

$$\lambda_2 = \left(1 - \frac{1}{n}\right)\left(1 - \frac{2}{n}\right) \qquad t_2 = \frac{1}{2}\mu_1 - \frac{3}{2}\mu_2 + \mu_3$$

$$\lambda_3 = \left(1 - \frac{1}{n}\right)\left(1 - \frac{2}{n}\right)\left(1 - \frac{3}{n}\right) \qquad t_3 = -\frac{n-1}{5n-6}\mu_1 + \frac{6n-7}{5n-6}\mu_2 - 2\mu_3 + \mu_4$$

If n is large, we may write

$$t_3 = -\frac{1}{5}\mu_1 + \frac{6}{5}\mu_2 - 2\mu_3 + \mu_4$$

Correspondingly, we have four equations for the moments in terms of the latent vectors.

$$\mu_1 = t_0$$

$$\mu_2 = t_0 + t_1$$

$$\mu_3 = t_0 + \frac{3}{2}t_1 + t_2$$

$$\mu_4 = t_0 + \frac{9}{5}t_1 + 2t_2 + t_3$$

In the zero generation, we have $_0\mu_n = q^n$ giving

$$_0\kappa_0 = q$$
$$_0\kappa_1 = -q(1-q)$$
$$_0\kappa_2 = q(1-q)\left(\frac{1}{2} - q\right)$$
$$_0\kappa_3 = -\frac{1}{5}q(1-q) + q^2(1-q)^3$$

Then for V_w in the r^{th} generation,

$$\begin{aligned}
{}_rV_w &= {}_r\mu_2 - {}_r\mu_4 \\
&= {}_r\kappa_0 + {}_r\kappa_1 - \left({}_r\kappa_0 + \frac{9}{5}{}_r\kappa_1 + 2{}_r\kappa_2 + {}_r\kappa_3\right) \\
&= -\frac{4}{5}{}_r\kappa_1 - 2{}_r\kappa_2 - {}_r\kappa_3 \\
&= -\frac{4}{5}{}_0\kappa_1\,\lambda_1^r - 2{}_0\kappa_2\,\lambda_2^r - {}_0\kappa_3\,\lambda_3^r \\
&= \frac{4}{5}q(1-q)\,\lambda_1^r - q(1-q)(1-2q)\,\lambda_2^r + \left[\frac{1}{5}q(1-q) - q^3(1-q)^3\right]\lambda_3^r
\end{aligned}$$

Now $\lambda_1^r = \left(1-\frac{1}{n}\right)^r$ and as $\frac{1}{n}$ is the expected relative decline in heterozygosis each generation, $\left(1-\frac{1}{n}\right)^r$ is the proportion remaining after r generations and is equal to $1-F$. If n is large, then $\left(1-\frac{2}{n}\right)^r = \left(1-\frac{1}{n}\right)^{2r} = (1-F)^2$. Thus $\lambda_2^r = (1-F)^2$ approximately and $\lambda_3^r = (1-F)^6$, giving

$$V_w = a(1-F) + b(1-F)^3 + c(1-F)^6.$$

Similarly,
$$\begin{aligned}
{}_rV_a &= 2({}_r\mu_3 - {}_r\mu_4) \\
&= 2\left[{}_r\kappa_0 + \frac{3}{2}{}_r\kappa_1 + {}_r\kappa_2 - \left({}_r\kappa_0 + \frac{9}{5}{}_r\kappa_1 + 2{}_r\kappa_2 + {}_r\kappa_3\right)\right] \\
&= 2\left(-\frac{3}{10}{}_r\kappa_1 - {}_r\kappa_2 - {}_r\kappa_3\right) \\
&= \frac{3}{4}a(1-F) + b(1-F)^3 + 2c(1-F)^6
\end{aligned}$$

V_t is given by the expression, $V_t = \mu_2 - \mu_1^2$
where $\mu_2 = q - q(1-q)(1-F)$
$= q^2 + q(1-q)F$, giving
$$V_t = q(1-q)[q(1+q) + F(1-2q^2) - F^2q(1-q)]$$

If q is small, this reduces to Fq. V_b is then obtained as $V_t - V_w$ but unfortunately no simple expression seems to exist. Expansion gives, in order of powers of F,

$$V_b = q(1-q)\left\{F4q^2 + F^2[14q(1-q) - 6q] + F^3[3 + 2q - 20q(1-q)]\right\} + \ldots.$$

If q is small, the first and second terms, being of the third and second order in q, will be small compared to the third term and therefore if F is small, $V_b = 3F^3q$.

For the genes showing overdominance, the general principles are the same except that the values for the first and third moments also enter into the calculation.

The variance between the performances of the lines on top-crossing to the original population can easily be calculated. If the gene frequency in a line is q_1 then the proportion of homozygous recessives in back-crossing to the original population (in which the gene frequency is q) is qq_1, and this will be the mean phenotype value of the cross. The variance required is then q^2 var $q_1 = q^2(\mu_2 - \mu_1^2) = q^2[q - q(1-q)(1-F) - q^2] = Fq^2(1-q)$ which increases as the first power of F. If we cross two lines in which the gene frequencies are q_1, q_2, the mean phenotypic value of the cross is q_1q_2. To calculate the total variance between such crosses, we have to find the variance of q_1q_2 when q_1 and q_2 are independent members of the q distribution. Actually the moments about zero of such a distribution of a product are the products of the moments of the parent distributions. As in this case, the two samples are from the same distribution, the moments about zero of the product distribution are the square of the moments of the q distribution. In terms of those moments the variance between line crosses $= \mu_2^2 - \mu_1^4$

$$= q^2(1-q)F(F + 2q - Fq)$$

Thus the variance between line crosses is proportional to F^2 if q is small.

THE INHERITANCE OF CERTAIN BLACK AND BROWN PIGMENTS IN THE SOYBEAN

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IN the soybean (*Glycine max*), two main types of pigment in the seed coat have been described: green and yellow pigments, and black and brown pigments. In the latter group, strains of soybeans are known with black, imperfect black, brown, light brown, reddish brown, and buff pigments. Certain types are also known with combinations of black and brown pigments.

NAGAI (1921) and OWEN (1927) have shown that the black coats are really an intense purple, due to an anthocyanin pigment, and that the brown shades are due to phlobaphene pigments.

Two additional types of coloration are known. One is the brownish pigment found as a smudge on the coats of varieties having black pod color. This appears to be an infiltration of pigment from the pod. OWEN (1928) has identified it as an oxidation pigment.

Another colored condition is a purple blotch known as "purple spot" disease and is caused by the fungus *Cercosporina kikuchii*. MATSUMOTO and TOMOYASU (1925) found the pigment responsible for this coloration to have properties similar to anthocyanins.

REVIEW OF LITERATURE

These studies are concerned with the group of black and brown pigments due to anthocyanins and phlobaphenes. A number of investigators have studied the inheritance of these pigments but there has been some disagreement in their interpretations and there is considerable confusion in the genetic symbols assigned. The writer believes it will be well to attempt to coordinate these reports in the light of his experiments. This report is concerned with the *type* of pigment present in the seed coat rather than its distribution. However, to clarify the subject for the reader, it should be mentioned that the distribution of pigment is conditioned by an allelic series. *I* acts to prevent the normal development of pigment in the seed coat; i^i restricts pigment to the hilum; i^k permits the development of a saddle pattern extending from the hilum and i permits full pigmentation.

Table 1 is a list of the types previously reported, together with several not so far reported. To identify the colors more certainly, RIDGWAY's (1912) designations have been included, together with the common names used by

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TABLE 1

Summary of symbols used by various authors to designate black and brown seed coat colors.

Nagai	Woodworth	Owen	Stewart	Williams	Ridgway's color	Common designation
C	H, (R_2T)	R_2T	R_2T	T	....	....
R	B, (R_1)	R_1	R_1	R	....	....
RC	HB, (R_1R_2T)	R_1R_2T	R_1R_2T	RT	Black	Black
rCO		r_1R_2T	r_1R_2T	rT	Dresden brown	Brown
rCo	....	....	$r_1{}^oR_2T$	r^oT	Liver brown	Red-brown
c		R_1R_2t	R_1R_2tW	RtW	....	Imperfect black
rc	....	$r_1r_2't$	r_1r_2t	rtW	Hazel	Buff
....		R_1r_2t	R_1r_2tw	Rtw	Hazel	Buff
....	....	....	....	rtw	Hazel	Buff
....	....	....	....	r^otW	Hazel	Buff
....	....	....	....	r^otw	Hazel	Buff

the investigators. The genetic symbols used by NAGAI, WOODWORTH, OWEN, STEWART, and those proposed by the present author are also included.

PIPER and MORSE (1910) first reported the dominance of black over brown but did not assign any symbols to the genes involved. They reported a simple 3:1 ratio in F_2.

NAGAI (1921) also reported black dominant to brown and brown dominant to reddish brown. He assumed three factors to account for the colors he found. *C* was present in blacks and browns, *c*, in imperfect blacks and buffs. *R* and *r* distinguished between blacks and browns or between imperfect black and buff, when *C* and *c* respectively were present. To differentiate between brown and reddish brown, he assumed a factor pair *O*, *o* which in combination with *Cr* gave brown and reddish brown, respectively.

WOODWORTH (1921) postulated two complimentary factors, *B* and *H*, to account for black pigment. The types *bbHH* and *BBhh* were considered to be brown. No attempt was made to distinguish shades of brown. He also postulated that the double recessive would be yellow since both recessives seemed to reduce the pigment. Later (1932) he reported that crosses between light brown hilum and dark brown hilum gave black hilum in the F_1. He assumed complete linkage between *H* and the gene *T*, for pubescence color. Types with *T* had tawny pubescence and dark brown hilum and those with *t* had gray pubescence and light brown hilum.

OWEN (1928) used the symbols R_1 and R_2T to account for his results. Black was given the formula R_1R_2T, brown r_1R_2T or $r_1r_2'T$, imperfect black R_1r_2tW. He found that in one cross, purple flower color (*W*) was necessary for the production of imperfect black.

STEWART (1930) agreed with OWEN in the formula for black, but showed that OWEN's assumption of an allelic series at the R_2 locus was not necessary, and further showed that OWEN's use of formulae was inconsistent and not in accord with the facts. STEWART assumed that both R_1r_2tw and r_1r_2tW would give buff coats and R_1r_2tW, imperfect black. He gave evidence of an allelic series at the R_1 locus, R_1T being black, r_1T brown, and $r_1{}^oT$ reddish brown.

EXPERIMENTAL DATA

Black × Brown

The writer is inclined to agree with STEWART's interpretation, but wishes to stress several points not brought out by former investigators. In the inheritance of black, brown, and reddish brown, all the writer's results have agreed with those of STEWART and support the idea of an allelic series at the R_1 locus. The following summaries of the F_2 3 : 1 ratios obtained by the writer serve to substantiate STEWART's allelic series.

		Black	Brown	Red-brown	chi²
Black (*R*) × Brown (*r*)	Actual	576	200	....	.2474
	Expected	582	194	....	....
Black (*R*) × Red-brown (r^o)	Actual	298	....	98	.0135
	Expected	297	....	99	....
Brown (*r*) × Red-brown (r^o)	Actual	....	335	111	.0030
	Expected	....	334.5	111.5	....

As will be seen in the above table, the actual ratios are very close to the calculated ratios. Only a multiple allelic series can explain these results. NAGAI had used *O, o* for brown and reddish brown, so STEWART used the superscript "°" to tie his r_1^o to NAGAI's results. Plants producing black, brown, or reddish brown seed may have either purple (*W*) or white (*w*) flowers.

Closely linked genes (R_2T) vs. a single gene, T

As mentioned above, NAGAI, WOODWORTH, OWEN, and STEWART have all postulated at least two factors as necessary for the full development of black and brown color. NAGAI used *R* and *C*, WOODWORTH suggested *B* and *H*, but OWEN and STEWART have postulated a third gene (R_2), closely or completely linked with *T* and complementary to R_1. It has been observed that in general, full colored blacks and browns have tawny pubescence as opposed to gray pubescence on imperfect black and buff types. There are, however, some blacks with gray pubescence, and for this reason OWEN and STEWART proposed the factor R_2 as complementary to R_1 and very closely linked with *T*, the factor for tawny pubescence. The black-seeded, gray pubescent types were then considered to be crossovers (R_1R_2t).

The writer prefers to drop R_2 and use only *T*, which has been assumed to be completely linked with R_2. WOODWORTH (1921) at first was inclined to this usage. He says, "As the linkage is complete between *H* (*now* R_2) and *T*, the combined effects of these two factors may be thought of as due to a single factor which has more than one effect. Thus, *T*, the factor for tawny pubescence, may be thought of as not only being able to change the pubescence color from gray to tawny, but also as acting with *B* (*now* R_1) to change the hilum color from brown to black. This is the most convenient way of regarding the situation."

In all the crosses studied in the soybean no instance of crossing over between R_2 and *T* has been reported. This fact casts considerable doubt on the existence of more than one gene.

The imperfect black type

OWEN and STEWART have mentioned an "imperfect black" type with black pigment and gray pubescence. In types where only the hilum is pigmented, the black pigment is confined more to the center of the hilum and is usually surrounded by a narrow buff band. Where the whole coat is pigmented, varying mixtures of buff and black pigment appear. Sometimes there is so little black that the coat appears to be almost buff, while in other strains, the black is so intense that the buff pigment is not noticeable. However, the black pigment is always much duller than the normal *RT* black. WOODWORTH (1921) had thought of this as a crossover type, R_1R_2t, but OWEN (1928) and STEWART (1930) give R_1r_2t as the formula for imperfect black. In the simplified system suggested above, imperfect black would have the formula *RtW*. That imperfect black is not a crossover type is indicated by the fact that it is distinct phenotypically from true black and that the imperfect black type is only developed in the presence of *W*. That imperfect black is really *R* can be proven by crossing it with black (*R*), and brown (*r*).

In crosses between imperfect black (*RtW*) and blacks of the composition *RTW*, black is dominant and only the parental types segregate in F_2. In crosses between imperfect black and brown (*rTW*), the F_1 is always pure black, indicating that the imperfect black must be *R*. The results of the writer all agree with previous work that imperfect blacks must have gray pubescence (*t*) and purple flowers (*W*).

The black-coated, gray pubescent types mentioned above are introductions from the Orient. If these are really crossover types of the formula R_1R_2t, then in crosses with ordinary gray pubescent types, only gray pubescent types should be produced in F_1 and F_2.

The results of a cross between T24A,[2] of the formula *rtW*, and T69 (P.I. 64698),[3] a black bean with a clear gray pubescence, as good a gray as T24A, indicate that T69 is not a crossover type. The formula of T24A has been proven in a number of crosses. The F_1 was black-seeded with gray pubescence, but the F_2 progeny consisted of 46 gray to 12 tawny plants. If this is really a 13:3 ratio, as it may well be, the results can be explained by assuming a dominant gene T_2 for gray pubescence, epistatic to T_1 for tawny pubescence. Since it is well established that T24A is *rt*, any gene for tawny pubescence must come from T69 which accordingly must be T_1. Then T69 would be T_1T_2 and T24A would be t_1t_2. If such is the case, it is evident that T_2 has no effect on seed coat color. At any rate, T69 can not be considered as a crossover type.

In view of these facts, and because it is the simpler explanation, the writer prefers to use only *T* as complementary to *R*. Possibly NAGAI's *C*, which is apparently identical with *T*, should be used because of prior usage, but since *T* indicates the effect on the pubescence color (from *tawny*) and

[2] The prefix "T" refers to type numbers in the file of the Plant Breeding Division of the Agronomy Department of the University of Illinois.

[3] The prefix P.I. indicates introductions of the Office of Foreign Plant Exploration and Introduction of the United States Department of Agriculture.

since NAGAI has made no mention of the effect of *C* on pubescence color (which is strange indeed, since he must have had the two pubescence colors segregating in some of his crosses where the effect of *T*, *t* on seed coat color is quite pronounced), the writer prefers to retain the present symbol *T*, simply dropping R_2 and assigning its properties to *T*. R_1 then becomes simply *R*.

Imperfect Black × Black

As mentioned above, crosses between purple-flowered black and imperfect black give 3 black to 1 imperfect black in F_2, since only *T* is segregating. However, in crosses between white-flowered black and imperfect black, both *W* and *T* are segregating. In the cross between Peking (*RTw*) and T24 (*RtW*) the following F_2 ratio was observed:

	Actual	Expected (9:3:3:1)	chi²
Purple-flowered black (*RTW*)	109	108	.4815
White-flowered black (*RTw*)	38	36	
Imperfect black (purple flowers) (*RTW*)	35	36	
Buff (white flowers) (*Rtw*)	14	12	

Flower color has no effect on seed coat color on RT plants but it does on Rt plants.

Imperfect Black × Brown

As mentioned above, the cross between imperfect black and purple-flowered brown (*rT*) gives black in the F_1. The cross between T116, a purple-flowered brown (*rTW*) and T24, an imperfect black, gave the following ratio in F_2:

	Actual	Expected (9:3:3:1)	chi²
Black (*RTW*)	78	76.5	.1569
Brown (*rTW*)	25	25.5	
Imperfect black (*RtW*)	24	25.5	
Buff (*rtW*)	9	8.5	

In the cross between imperfect black and white-flowered brown (*rTw*), we have all three genes segregating. The F_1 is black with tawny pubescence and purple flowers. The F_2 of the cross between T24 (*RtW*) and Harbinsoy (*rTw*) gave the following results:

	Actual	Expected (27:9:9:9:3:3:4)	chi²
Black pigment, purple flowers (*RTW*)	66	67.5	.221
Black pigment, white flowers (*RTw*)	23	22.5	
Imperfect black pigment, purple flowers (*RtW*)	24	22.5	
Brown pigment, purple flowers (*rTW*)	22	22.5	
Brown pigment, white flowers (*rTw*)	8	7.5	
Buff pigment, purple flowers (*rtW*)	7	7.5	
Buff pigment, white flowers (*Rtw* and *rtw*)	10	10.0	

The buff types

The three crosses just mentioned have each produced some buff-colored types. It will be noted that in the first of these, the gene *W* is responsible for the difference between imperfect black and buff and in the second cross *R* is responsible. In the third cross, both genes are segregating. It is evident that there are two genetic types of buff, indistinguishable phenotypically.

In table 1 it will be seen that these two buff types, *rtW* and *Rtw*, have been reported before. It is interesting to note that apparently *R* and *W* can be substituted in this case and give the same effect on the seed coat. This is not strange when it is recalled that the gene *W* produces anthocyanin pigment in both the flower and the stem. The buff hilum and buff types of STEWART were all of the *rtW* type, and those of OWEN also were *rtW*, except C3 and its segregates. Most of WOODWORTH's buff (light brown) hilum types were of the *Rtw* type. Illini, Wea, Dunfield, Mukden, Blackhawk, and many white-flowered, gray pubescent grain varieties are of this type. Mandarin and Habaro are of the *rtW* type.

That the types identified as *rtW* and *Rtw* are really such can be proven by crossing them. If the assumption is correct, the F_1 should have imperfect black pigmentation (*RrttWw*). In the cross between T122 (*Rtw*) and T24A (*rttW*), the F_1 was imperfect black as expected.

The F_2 of this cross gave the following results:

	Actual	Expected (9:3:4 basis)	chi²
Imperfect black (*RtW*)	206	202.5	.1642
Purple-flowered buff (*rtW*)	65	67.5	
White-flowered buff (*Rtw*) and (*rtw*)?	89	90.0	

This ratio is evidently a 9:3:4 ratio, indicating that the triple recessive (*rtw*) is a buff indistinguishable from *Rtw*.

The identification of the *rtw* type was of special interest. Since the recessive alleles of all three genes seem to act to reduce pigmentation, it was wondered if the triple recessive might be devoid of black and brown pigment, *i.e.*, a recessive yellow.

In this cross we should expect 22 plants of the triple recessive (*rtw*). If these were yellow they would have been easily distinguished, but no yellow-seeded plants occurred.

WOODWORTH (1921) postulated that the combination $r_1(r_2)t$ would be yellow or colorless due to the fact that both recessives reduce the pigment. In a cross between P.I. 20406, a colorless hilum yellow, and P.I. 20854, black, he considered the F_2 ratio to be a 9 black : 6 brown : 1 colorless (r_1r_2t) but since environmental causes made all the seed coats mottle with streaks of pigment, he was unable to distinguish the latter classes and recorded instead a 9:7 ratio.

The writer would prefer to ascribe the colorless hilum of P.I. 20406 to the gene *I* for inhibition of pigment since in all crosses at the Illinois Station, this variety (named Elton) has behaved as if of the constitution *RtI*.

In order to positively identify the triple recessive, another cross was made. In this, T91 (*rTw*) was crossed with T24A (*rtW*). In this cross only *T* and *W* were segregating. Since both parents were homozygous *r*, any white-flowered, gray pubescent plant would be of the type *rtw*. Actually, there were three such in a population of 95 plants. All of these showed the buff color and were indistinguishable from T24A (*rtW*), in color of coat. These experiments would indicate then that the dominant alleles of the genes *T*, *W* simply add something to a base color of buff rather than that the recessive allelomorphs remove color, or else that the chemical effect of these genes on seed coat color is almost identical.

The writer has added several other new types. Previous writers had made no mention of what the r^o combinations with *t* might be. In the cross between P.I. 82235, a reddish brown, tawny pubescent type (r^oTW), and P.I. 82273, a buff with purple flowers and gray pubescence, the F_1 was phenotypically r^oTW and the F_2 segregated in a simple ratio of 3 reddish brown : 1 buff, showing that P.I. 82273 is really an r^otW buff. The actual figures were 29 r^oTW : 10 r^otW.

The triple recessive type r^otw has not previously been mentioned. This type was obtained from a cross between Ogema (r^oTw) and P.I. 82273 (r^otW). The F_1 was reddish brown like the Ogema parent but had purple flowers. The F_2 segregated as follows:

	Actual	Expected (9 : 3 : 3 : 1) basis	chi²
Purple-flowered, red brown (r^oTW)	123	121.5	.0988
White-flowered, red brown (r^oTw)	39	40.5	
Purple-flowered, buff (r^otW)	41	40.5	
White-flowered, buff (r^otw)	13	13.5	

Since all *T* plants were red brown (r^o) it is evident that there was no segregation at the *R* locus, so all the buff-seeded plants must be r^ot. The white-flowered, buff-seeded plants must then be r^otw. These were indistinguishable in color from r^otW buffs.

Crosses between buff and other colors

Since there are five genetic types of buff and the combinations of these with black, brown, reddish brown, and imperfect black are too numerous to report in detail, no attempt will be made to illustrate these crosses.

It has been mentioned above that crosses between *Rtw* buff and *rtW* or r^otW buffs will give imperfect black in F_1 but other crosses between buffs will give only buff in F_1 and F_2.

SUMMARY

The somewhat confused literature on the inheritance of black and brown pigments in the seed coat of the soybean has been summarized and coordinated. Three new genetic types of buff are reported. Black, brown, and reddish brown are due to an allelic series, *R*, *r*, r^o. These colors are developed

only when combined with *T*, for tawny pubescence. In combination with *t* (gray pubescence), they give imperfect black, buff, and buff, respectively. The imperfect black type only develops in combinations with *W'* for purple flower color. All white-flowered, gray pubescent types have buff pigment in the coat unless inhibited by some member of the *I* series. There are five genetic types with buff pigment, indistinguishable phenotypically.

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